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Nuclear and cell division in Nitella and Chara[1]

JOHN S. KARLING

(WITH SIX TEXT FIGURES AND PLATES 10–13)

INTRODUCTION

The uncertain taxonomic position of the Characeae in the plant kingdom has made these plants the objects of interest to botanists for many decades; and by reason of their relatively simple and diagramatically regular habit of growth as well as the ease with which they may be grown and handled under laboratory conditions, they have been the subject of numerous physiological, morphological, and cytological investigations. Notwithstanding these extensive studies, a review of the literature as to the general cytology of the group shows many differences in observation and interpretation concerning the presence of centrosomes, the method of formation of the spireme and chromosomes from the resting nucleus, cell plate formation, and the nature of the chromatic bodies in the cytoplasm about the nucleus in the resting condition and during mitosis. Most of the cytological work has referred to various species of the genus *Chara*, while comparatively little study has been devoted to *Nitella* and the other genera of the Characeae.

Nägeli (51), Schmitz (69), Treub (81), Strasburger (74) and Johow (31) were among the first investigators to describe the nuclei and nuclear and cell division in the *Characeae*. As early as 1844, Nägeli (51) described the nuclei of *Nitella flexilis*, *Chara vulgaris*, *C. hispida*, and *C. gracilis* as consisting of a clear, viscid, seldom granular nuclear vesicle and a nucleole. Schmitz (69, p. 367) states that the nuclei in *Chara* multiply in the same manner as the nuclei of higher plants, but he gives no drawings.

[1] Contributions from the Department of Botany of Columbia University, no. 344.

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Treub describes "nuclear fragmentation" and cell plate formation in cells of the antheridial filaments, one-celled antheridium, and terminal leaf cell of *Chara fragilis*. He finds that cell division is accomplished by the centrifugal growth of a cell plate instead of by furrowing or constriction. Shortly afterwards, Strasburger (74, pp. 194–196, *figs. 48–52. pl. 13*) figured spindles, chromosomes, daughter nuclei and cell plates in the nodal cells of *Chara foetida*. Johow rejects the views of the previous investigators, and maintains that nuclear and cell division in *Chara foetida* are in no way comparable to these processes in the higher plants. He describes the nuclear membrane as disappearing, leaving the chromatin bodies lying free in the cytoplasm where they begin to multiply. They later separate into two masses, and finally the small, granular bodies unite to form larger bodies. The membranes of the two daughter masses appear shortly afterwards, forming two nuclei. Nowhere in this process do spindle fibers appear, and the cell plate, arising between the daughter nuclei, appears in optical section as a double row of granules.

Zimmermann (88) believes that the nucleoli of the *Chara* nucleus fragment, pass out into the cytoplasm of the cell and are latter aggregated in the daughter nuclei. From his observation of the nuclei of unsectioned apical cells and eggs of *Chara aspera*, *C. jubata*, and *Nitella syncarpa*, Overton (57) believes that the nuclei possess no true nucleoli, but large, irregular chromatic bodies. In 1893 Schottländer (70) described centrosomes in the resting vegetative cells and in all stages of division in the antheridial cells of *Chara foetida*. In the resting stages two centrosomes are found lying on the nuclear membrane surrounded by a clear space, and as the nucleus goes into the spireme stage they begin to move apart. This continues until the centrosomes lie at the opposite poles of the nucleus.

Migula (44, pp. 52–53) describes indirect nuclear division in the apical and nodal cells of *Chara hispida*. In this species he claims to find more than one nucleus in each cell, but is not certain whether these bodies have arisen by direct or indirect division. Belajeff (4) describes the formation of chromosomes, spindles, and cell plates in the division of the antheridial filaments of both *Nitella* and *Chara*. He figures the chromosomes in *Chara* as small, round granules, and in *Nitella* as slender

filaments. He is not certain, however, about the presence of attraction spheres or centrosomes, but he describes small, deeply stained bodies lying on the nuclear membrane. The view of Schottländer as to the presence of centrosomes is confirmed by the observations of Kaiser (31) on dividing cells of three species of *Chara* and two species of *Nitella*. He finds centrosomes in the resting cells as well as in all stages of nuclear division. The centrosomes lie close to the nuclear membrane in pairs surrounded by a clear region, but no astral rays are figured.

The most complete account of mitosis in the Characeae is that of Debski (10, 11), who gives a considerable series of successive stages in the division of the nucleus and cell of *Chara fragilis*. As to the presence of centrosomes, Debski (10) was apparently uncertain, although in his summary he states that they are not present in the Characeae. In some preparations he saw centrosome-like bodies, but he was not sure whether they occur regularly. The first attempt at a cytological study of fertilization was by Götz (26). De Bary (9) had observed the entrance of the antherozoids into the oogonia several years before, but had not been able to see the behavior and fusion of the gamete nuclei in the egg. Götz figures dividing cells and nuclei in the development of the oogonium of *Nitella flexilis*, *N. opaca*, and *Chara foetida*, but he did not observe the presence of centrosomes.

Among the later cytological studies on the Characeae are those of Ernst (18), Oehlkers (55), Mirande (45), Riker (61), Mangenot (40) and Tuttle (82). Ernst has contributed much to our knowledge of the morphology and chromosome numbers of the dioecious, and, what he terms parthenogenetic forms of the Characeae. His observations will be referred to in more detail later. Oehlkers studied nuclear division in the germination of the oospore of *Chara fragilis*, *C. foetida*, and *Nitella syncarpa*, and holds that the reduction in chromosome number occurs in the first division of the nucleus of the germinating zygote, thereby confirming the claims of Debski (11) and Götz (26) that no reduction takes place at the time of the so-called gametogenesis. Directly opposed to this view are the observations of Tuttle, who maintains that reduction occurs in the first division of the cells which become the antheridia and oogonia. According to Tuttle, the *Nitella* and *Chara* plant as commonly seen in

nature belongs to the sporophyte rather than the gametophyte generation. The observations of Mirande, Riker, and Mangenot deal with mitochondria and plastids in the cells of Characeae, and will be considered in connection with these bodies.

MATERIALS AND METHODS

The following observations were made on plants of *Nitella gracilis* Smith, secured from one of the small ponds at Woods Hole, Massachusetts, *Chara coronata* Ziz. from Greenwood Lake, New Jersey, and an unidentified species each of *Chara* and *Nitella* growing in a shallow lake in Van Cortlandt Park, New York City. In an earlier paper (32) I have referred to the *Chara* species as *C. fragilis* Desv. because of its close resemblance to this form, but its identity has not been definitely established.

The plants were grown in tap water with sand loam as a substratum in large battery jars in north, south, and east windows of laboratories where the temperature was approximately 20° C. Vegetative growth in the battery jars was vigorous, some of the plants in the *Chara* cultures reaching a length of three feet. *Nitella gracilis*, *N. sp.*, and *Chara coronata* have fruited continuously in the laboratory for three years without regard to time of year. The unidentified species of *Chara* referred to as *C. fragilis* and reported by the author in an earlier paper as forming no reproductive organs under laboratory conditions from September, 1923, to May, 1924, has subsequently, 1924–1926, been found to produce a few antheridia and oogonia in winter under ordinary laboratory and greenhouse conditions. As reported, when the normal daylight period was supplemented with electric illumination, reproductive organs were produced in abundance during the winter months.

For the study of fixed material, Flemming's strong, medium, and weak chrom-acetic mixtures, Bouin's and Merkel's solutions, chrom-acetic acid, Allen's modification of Bouin's fluid, and Carnoy's, Benda's, Regaud's, and Němec's fluids were used as fixing agents. Merkel's and Flemming's solutions gave the least shrinkage and the best division figures. Heidenhain's iron-alum haematoxylin gave good results for the study of the chromosomes and the centrosome question, but Flemming's triple stain gave a finer differentiation of the nucleus and cell in general.

A careful comparison of the fixed and stained preparations

was made with cells mounted directly in water and sugar solutions, and with preparations stained *intra-vitam* with aceto-carmine, according to the method used by Belling (5) and Sands (66). The apical cells and the primordia of the antheridia and oogonia of *Nitella* are very favorable for such study before they become filled with chloroplasts and starch. Even more favorable are the cells of the antheridial filaments. These cells are free from chloroplasts and stored foods, and their relatively large nuclei are not obscured by strands of streaming cytoplasm and large vacuoles, such as are present in the stamen hairs of *Tradescantia* and the root tip cells of *Allium* and *Vicia*. Moreover, the entire processes of nuclear and cell division from the resting stage to the reorganization of the daughter nuclei and the formation of the new cell wall may often be seen in a single filament. A complete series of adjacent, successive stages of nuclear and cell division from the apex to the base, or vice versa, is not uncommon in the same filament. Frequently, long filaments may be found in which as many as fifty cells are undergoing division at the same time.

The method of preparing the living and aceto-carmine mounts of antheridial filaments was as follows: After removing the antheridia from the plants and mounting, the shield cells were peeled off with fine steel needles, exposing the manubria, capitula, and the filaments. The antheridial filaments were then carefully separated under the low power of the microscope with as little injury as possible. As soon as the cover glass was added the cells were ready for study.

The cells of the antheridial filaments are very sensitive to mechanical injury, and too much care cannot be used in selecting uninjured filaments for study. A slight injury to a cell will have a marked effect on the position and appearance of the nuclei in all cells of the filament. This effect is clearly shown in figure 51 of a filament injured a short distance from the apex. In this figure the nuclei have moved towards the cross wall nearest the injury. The nuclei in the cells near the apex lie against the basal cross wall, while those below the injury lie against the upper cross wall. Not only is the position of the nuclei modified by the injury, but their shape and appearance as well. The nuclei adjacent to the injured cell are very much flattened and seem denser and more compact in appearance. The change in ap-

pearance and position of the nuclei in this figure seems to be a general reaction of the nuclei of antheridial filaments to injury. Numerous long filaments have beeen found in which marked changes in shape, appearance, and position were visible in nuclei that were thirty cells removed from the injury.

This reaction of nuclei to injury (described for figure 51 of *Chara*) is wide-spread among plants and has long been known and figured extensively. Tangl (78), Nestler (54), Miehe (43), Schürhoff (71), Hottes (30) and Němec (53) observed that injury to a cell or group of cells in the roots and epidermis of various plants resulted in a general movement of the proptolasm as a whole towards the region of injury. Nestler found that the effect of injury to a cell could be detected by changes in the position of the nuclei in a radius of .7 to .8 mm. of the injury. The reaction is often so strong that the nuclei even pass through the cell wall nearest to the injury (Miehe, 43, *figs. 2, 3, 4*). Migration or extrusion of chromatin from the nuclei of pollen mother cells into the cytoplasm of adjacent cells in segments of anthers is a general occurrence, according to Körnicke (36), Farmer and Digby (19), Gregory (27), Digby (15, 16, 17), Rosenberg (62, 63), Gates (23), Fraser (22), Derschau (14), West and Lechmere (84) and Sinoto (73); and, although there is considerable controversy among these investigators as to the cause of this phenomenon, Körnicke and Sinoto (p. 109) believe that it is a direct response to certain traumatic stimuli, such as pressure and external injury to the anther.

NUCLEAR AND CELL DIVISION IN FIXED MATERIAL

Resting nucleus

In fixed and stained preparations the resting nuclei of the apical, nodal, and antheridial cells and eggs usually lie in the center of the cell, surrounded by the more or less homogeneous cytoplasm. The most striking feature of the nucleus of fixed preparations is the large nucleole-like body of highly stainable material at its center. This nucleole-like aggregate in *Nitella* and *Chara* appears to be similar to that described for fixed material of many of the Conjugatae, where the chromatin has been claimed to be aggregated into a compound nucleole-like body. In *Nitella* and *Chara* this body is large in proportion to the size of the nucleus, and in most cases appears to consist of a number

of smaller bodies. Frequently it lies in a large, clear space, but whether this is normal is questionable.

The small bodies making up the compound nucleole-like structure are often massed closely together, giving it an irregular outline and shape with light and dark regions throughout, as shown in figure 20. In some preparations where there has been slight plasmolysis the nucleole-like body is very dense and compact, but in properly fixed material the small bodies of which it seems to be composed may be entirely separate from each other. Many nuclei have been found in which the central region is filled with a large number of globular and angular, highly stained bodies. In the nucleus of an antheridial cell shown in figure 30 there are nine relatively large, round bodies and numerous smaller ones. Schottländer (*figure 36*) shows a similar nucleus of *Chara foetida* with five large bodies. In figures 29 and 31 a number of small bodies are grouped about a larger, central body. Figures 2, 3, and 5 show egg nuclei with large aggregated masses of deeply stained material in the center. In figures 2 and 3 a round body may be seen lying to one side of the mass. It appears vacuolated and has the appearance of a true nucleole. In figures 5 and 7, nuclei of an egg and apical cell, a peculiar modification is shown in which the compound mass has a number of knob-like projections. Figure 4 shows the nucleus of a nodal cell with four large round bodies in the center, and in figure 6, seven relatively small bodies are grouped in a clear central region of the nucleus. Strasburger (77, *figs. 2, 2b*) shows seven large, round bodies in nuclei of nodal cells of *Nitella syncarpa*. In addition to the mass of highly stainable material at the center, resting nuclei have been found with a large number of round and angular bodies in the nuclear network, such as are shown in figures 7 and 31.

Besides the granules and bodies described above, the resting nucleus shows a rather delicate chromatic reticulum or network. In most fixed preparations very little chromatin in the form of granules is visible in the reticulum. In the nuclei of figures 2, 3, 4, 5, 6, no definite granules are shown, but darker staining masses are visible in the reticulum, which appear as faint net-knots rather than definite bodies. Such a condition is not the universal rule, however. Many nuclei may be found with numerous dark staining granules and bodies in the nuclear network,

such as are shown in figures 7, 32, and 33. In the triple stain these bodies stain a faint violet in contrast to the deep red of the nucleole-like masses.

Whether the entire aggregate of highly stainable material at the center of resting nuclei in fixed preparations is to be regarded as a compound nucleole seems questionable. In many preparations, such as those shown in figures 2, 3, and 38, a round body that has all the appearances of a true nucleole may be found lying close to or in the center of the deeply stained mass of material. In such preparations the appearance is as though the chromatin material has aggregated into larger bodies around the nucleole. I have studied the same stages in unfixed and unstained and aceto-carmine preparations with the view of determining the nature of these chromatic masses. The nuclei of such preparations have a very different appearance from that shown in figures 1 to 7 and 29 to 34 of the fixed and stained material. No aggregate mass of bodies in the center is visible. Figure 8 shows an *intra-vitam* stained nucleus of an apical cell in which a single nucleole and numerous minute granules or bodies are visible, but no aggregate mass is present.

Prophase

As the nucleus goes into the early prophase of division, an increase in size occurs, accompanied by a marked change in the appearance of the nucleus. In fixed preparations the delicate nuclear reticulum appears to gradually disappear, and the nucleus becomes filled with deeply stained, irregular strands and bodies. The strands are finally transformed into a thin, irregular, discontinuous spireme. The stages of this process are shown in figures 32 to 37. The nucleus shown in figure 33 has a few chromatin granules distributed in the nuclear reticulum in addition to two small nucleoles. Later stages are shown in figures 34 and 35, where short irregular bodies and strands of chromatin are shown in the nuclei. In figure 35 the nuclear reticulum has almost disappeared, and the nucleus seems to be filled with deeply stained angular bodies and strands of chromatin. The general appearance up to this stage suggests that the small net-knots and granules have increased in size and fused to form larger fragments and strands. A further concentration of the chromatin into strands and threads continues until the

condition shown in figures 36, 37, 39, and 40 is reached, where the nucleus is filled with an irregular, convoluted, discontinuous spireme.

The presence of the compound body of highly stainable material at the center of resting nuclei in many members of the Conjugatae has led investigators of this group to the view that the spireme and chromosomes come wholly or partly from the nucleole. Moll (49), Mitzkewitch (46), Karsten (34), Bergh (6), and Van Wissenlingh (83) describe the origin of the spireme and chromosomes of *Spirogyra* from the nucleole, and Merriman (41), in *Zygnema*, Wolfe (85), in *Nemalion*, and Golenkin (25), in *Sphaeroplea*, report a somewhat similar condition in these forms. This much debated question as to the possible origin of the chromosomes from a chromatin nucleole has also arisen in connection with the nuclei of *Nitella* and *Chara*. Riker (61) believes that part of the nucleolar content of the nuclei in *Chara fragilis* and *C. verrucosa* flows out into the spiral, spireme threads. If the bodies and granules aggregated in the center of the nucleus in fixed preparations are the chromatin granules fused to form larger bodies, this view is untenable. The spireme would then not arise from the nucleole but from the chromatin aggregated about it. I have been unable to find evidence in fixed and stained material for the formation of the spireme and chromosomes from the so-called compound nucleole, or the fragments and bodies in the nucleolar region. In the early prophase of fixed and stained preparations the nucleole-like body generally appears to loosen up. In figures 34 and 35 no large nucleole is visible, but a number of irregular fragments may be seen among the chromatin strands. On the other hand, the nucleole is still present in the late spireme stages shown in figures 9 and 12. A large number of nuclei similar to these have been found. In figure 11 a number of spireme threads are closely grouped around the nucleole, but do not appear to be arising from this body.

Figure 9 shows a nucleus in which the spireme has segmented and begun to thicken into chromosomes, and still a compound mass of deeply stained material is present. Somewhat similar conditions are figured by Debski (10, *figs. 3, 4, 6*) in *Chara fragilis*. However, it is apparent from the diminished size of the nucleole-like body in figures 10, 11, and 12 that it loses

some of its material during the prophases. As stated above, and shown again in figure 8, no such aggregated mass of material is visible in the resting nuclei of living cells and aceto-carmine preparations. In figure 8, a drawing from an aceto-carmine preparation of an apical cell, the nucleole appears as a round body, and the structure of the nucleus as a whole has a coarsely granular appearance. In prophase stages of aceto-carmine preparations correspondong to those shown in figures 95, 96, 97, and 98, a round nucleole is frequently to be seen. While the evidence from fixed and stained material that the spireme and chromosomes do not arise from an aggregated nucleole-like mass at the center of the nucleus is not altogether convincing, the conditions described in living material and aceto-carmine preparations, it seems to me, indicate that these structures arise from an irregular granular chromatin reticulum distributed more or less evenly in the resting nucleus. The condition as I find it in *Nitella* and *Chara* is similar in many respects to that described by Lutman (39) for *Closterium*.

In fixed and stained preparations the early spireme appears convoluted and discontinuous, and seems to completely fill the nucleus. Debski (10) describes a continuous spireme in *Chara*, but I have not been able to determine this for *Nitella* or *Chara*. In many nuclei of fixed preparations the spireme appears coarsely granular and irregular. Figures 9, 10, and 38 show irregular spireme filaments in nuclei of *Chara*, while in figure 11 of *Nitella* is shown a nucleus in which the spireme is relatively smooth and uniform in outline. This difference in appearance of the spireme in the two genera is not, however, as marked as in figures 9, 10, and 11. In the early stages of the spireme it is difficult to find split threads. In some preparations of *Chara* I have seen what appeared to be a clear central line in the spireme, but the longitudinal splitting cannot be recognized with certainty until the segments have shortened into chromosomes and passed into the equatorial plate and metaphase stages of division. However, figure 38 shows a nucleus in which a number of segments appear double or longitudinally split. The three segments in this nucleus that show a longitudinal split have also alternating thick and thin places that suggest the appearance of chromomeres. Several of the other segments in this nucleus show what look like vacuoles in a median position. This figure is in many

respects similar to one of Strasburger's (77, *fig. 7*) of *Nitella syncarpa*, in which he shows a number of split segments. Debski (10) was unable to determine the time at which the spireme segments split in *C. fragilis*, and believes that no preparation for the halving of the chromosomes is visible until metaphase. The segments of the spireme continue to thicken and shorten to form the definite chromosomes. In figure 12 is shown a late prophase nucleus of *N. gracilis* in which 32 segments are visible. The nucleole is still present, but relatively small in comparison with its size in resting nuclei. There is no question that in this nucleus it is a single, rounded body and not an aggregation of smaller ones. Figure 9 shows a nucleus of *Chara sp.* with a number of thick irregular segments. In this nucleus a dense mass of intensely stained material is still present, which is similar in many respects to the aggregated masses in resting nuclei.

In fixed material the cytoplasm surrounding the nucleus in the spireme stage often appears very dense and shows a more or less radial arrangement. In figure 11 is shown a cell in which this arrangement of the cytoplasm is faintly visible. On the other hand, such a condition is not clear in figure 12. Such a preparation as the one shown in figure 11 suggests that the spindle may arise from radially arranged strands of cytoplasm about the nucleus, as described for pollen- and spore-mother cells. In cells of the antheridial filaments I have observed the condition shown in figures 39 and 40. In figure 39 the cytoplasm is denser at the two poles of the nucleus, and in figure 40 faintly differentiated fibers may be seen. There is some evidence for the presence of polar caps in these cells, but it cannot be said with certainty from these preparations that this is the regular mode of origin of the spindle in *Nitella* and *Chara*. When the spindle is fully developed it is usually a broad diarch to bipolar structure. Its shape varies considerably in different cells. In the antheridial filaments it is generally broad in shape, as shown in figures 49 and 50, but often it may be barrel-shaped with the fibers converging to the two poles (FIGS. 41, 46, 47 and 48). In the cells of the antheridial filaments the whole spindle figure may lie parallel, diagonal or perpendicular to the long axis of the filament. In apical and nodal cells as well as those from which the antheridia and oogonia are developed, the spindle is generally barrel-shaped.

As previously noted in figure 38, I have observed what appeared to be a clear central line in the chromosomes of a few nuclei in late prophase of fixed material. When the chromosomes become arranged on the spindle the longitudinal split becomes more evident. This longitudinal split is shown in figures 16a and 16b. I have not been able to see this in living material and aceto-carmine preparations, although some of the chromosomes in the cells marked *A* and *B* in figure 105 show a light median line. However, I do not regard this as an actual split in the chromosomes, but rather as a high light effect caused by round objects when they are photographed. Seen in polar view in fixed preparations, the chromosomes are often well separated and can be counted with readiness. I have made numerous counts of twelve chromosomes in *Nitella sp.*, and the form of *N. gracilis* with which I have worked has 34. Figures 13, 14 and 15 of *N. gracilis* show polar views of chromosomes and an antheridial cell, nodal cell, and an antheridial filament cell. Figure 13 shows the one-cell stage of an antheridium mounted and stained *in toto* in aceto-carmine. In addition to the 34 chromosomes in this figure may be seen six granules in the cytoplasm on one side of the group and one on the opposite side. The number of chromosomes in *Chara coronata* and *C. sp.* has not yet been determined, but in numerous polar views of the latter I have counted as many as 40 chromosomes.

Comparatively little study has been given to the problem of chromosome number in the various species of Characeae. Ernst (18) reports 12 chromosomes in *Chara crinita*, *C. galioides*, and *C. aspera*, and 24 in *C. crinita* var. *bivalens*. Strasburger (77) finds 18 in *C. crinita* and *C. fragilis*, and 12 in *Nitella syncarpa*. In *C. foetida* Schottländer reports more than 19 chromosomes, Götz (26) finds 16 to 18, and Oehlkers (55), 16. Debski (11) and Oehlkers report 24 chromosomes in *C. fragilis*, but Riker (61) later finds only 16 in the same species. Oehlkers confirms Strasburger's (77) count of 12 chromosomes in *N. syncarpa*. It is evident from these conflicting reports either that the chromosome counts have been incorrect in many instances or that varieties and forms of the same species exist with different chromosome numbers. The latter view is highly plausible, since a large number of varieties and forms are found in species of *Nitella* and *Chara* that differ considerably in morphology.

My own observations on *N. gracilis* do not confirm the results of Tuttle (82) who finds a reduction in chromosome number occurring in the first division of the cells that become the antheridia and oogonia in the species of *Nitella* which he studied. Figure 13 shows a polar view of a division figure in an antheridium in the one-cell stage. While there appears to be some evidence of pairing of the 34 chromosomes in this figure, no reduction occurs in this division, since the same number of chromosomes may be found in the cells of the antheridial filaments (FIG. 15). According to Tuttle's view, the division shown in figure 13 is the reduction division. Figure 15 shows 34 chromosomes in a cell of an antheridial filament consisting of approximately 30 cells. At least 36 divisions intervene between this polar view and that shown in figure 13, but no reduction in chromosome number has occurred. Figure 14 shows 34 chromosomes in a polar view of a division in a nodal cell. According to Tuttle's view that the *Nitella* plant as commonly seen growing in nature belongs to the sporophyte generation, we should expect to find in this figure double the number of chromosomes present in figure 15 of an antheridial filament. I have likewise counted 34 chromosomes in polar views of divisions in the 4, 8, and 16-cell stages of antheridia.

The chromosomes in the species I have studied vary considerably in size. They appear as long and short rods, wide V's and U's, and hook-shaped figures, as shown in figure 14 of *N. gracilis*. It is difficult if not impossible to classify them according to size. In figure 14 there are approximately 14 small, 15 intermediate, and 5 large chromosomes. Such a classification, however, can only be approximate, since the variation in size is so gradual. The large chromosomes labeled *o* in figure 14 can readily be recognized in the divisions of the vegetative cells. In figure 17 they are very conspicuous in the anaphase group of chromosomes. In *Nitella sp.* there are ten long approximately equal and two short chromosomes. As shown in the cell marked *A* in figure 105, the longest chromosomes in the equatorial plate stage may extend from the equatorial region almost to the ends of the cell.

An unusual variation in the size of the chromosomes in different cells has frequently been noted in the antheridial filaments of *Chara sp.* In numerous long filaments the chromosomes

have all appeared as round bodies or very thick rods more or less uniform in size, while in other filaments of the same antheridium they have shown the same variation in size and shape shown in figure 42. I have observed this condition both in fixed and aceto-carmine preparations. Figure 104 shows the chromosomes as round bodies grouped in the center of the cells. In the division figures shown in figures 106 and 110 the chromosomes are considerably longer and more variable in size. This contrast is further shown in figures 42 and 43. In figures 42, 44, 45, 46 and 47, the chromosomes are variously shaped rods, while in figure 43 they are scarcely twice as long as round. I have not observed such a marked difference between the chromosomes in various cells of the antheridial filaments of *N. gracilis*, however, but the chromosomes shown in figure 15 are very short, thick rods in contrast to the various sizes and shapes present in figures 41 and 46.

Belajeff (4) describes the chromosomes of *Nitella* as slender filaments, and those in the antheridial filaments of *Chara* as round granules. With the exception of such cells as those shown in figures 40 and 104, the chromosomes of my preparations of *Nitella* and *Chara* are of various sizes and shapes. Debski (10) has figured the chromosomes of *C. fragilis* as slender and long drawn out. The figures of Kaiser (32) are too small to show the relative shape and sizes of the chromosomes of the forms which he studied. Strasburger (75) describes the chromosomes of *C. fragilis* as long, variously-shaped structures that are closely grouped together. Ernst (18) reports that the chromosomes in the antheridial filament cells of *C. crinita* are somewhat longer than those in the vegetative cells.

Metaphase, anaphase, telophase

In the metaphase of fixed and stained preparations the chromosomes are arranged in the manner shown in figures 16a, 16b, and 46. In antheridial filaments the equatorial plate may extend completely across the cell. In most cases the chromosomes of such cells are too numerous and large to find space in the short diameter of the cell. The equatorial plate may then lie in a diagonal position, and not infrequently parallel to the long axis of the cell, as shown in figures 44 and 106. In metaphase the so-called split of the chromosomes becomes evident.

In figures 16a and 16b are shown chromosomes with a definite longitudinal split. The split appears to begin in a median or sub-median position and extends gradually to the ends of the chromosomes. The halves generally move apart in the shape of V's and U's, the ends remaining longest in contact. In figures 45 and 46, numerous wide V's and U's are shown. The shape of the chromosomes in late metaphase indicates a median and sub-median fiber attachment, but numerous cells have been found where the fiber attachment to the chromosomes is atelomitic and telomitic as well. In figure 17, the large chromosomes are hook-shaped at one end, suggesting the atelomitic attachment.

As the chromosomes move towards the poles in the anaphase, they are generally V, U, and hook-shaped, with the bend towards the poles of the spindle. This is shown in figures 17, 18, and 47. In cells of the antheridial filaments where the equatorial plate lies diagonally in the cell, as early observed by Treub (81) and Giesenhagen (24), and shown in figures 41, 110, and 111 of my preparations, or parallel to the long axis of the cell, as in figures 44 and 106, the spindle and chromosomes apparently rotate in the cell during anaphase and early telophase, so that the cell wall which is to be formed later between the daughter nuclei will divide the cell transversely. In cells where the equatorial plate lies approximately parallel to the long axis of the cell, as in figures 44 and 106, the rotation must of course be approximately 90 degrees in order that a transverse wall may be formed. It sometimes happens that the rotation of the division figure is not completed before the daughter nuclei are formed and the new cell wall begun. In such cases a corresponding modification of the position of the new wall follows. The separation of the chromosomes and their movement to the poles in *Nitella gracilis* is generally regular, but occasionally "lagging" chromosomes are visible on the spindle (FIG. 18).

The fibers which appear attached to the chromosomes in fixed and stained preparations are very delicate but distinct. The central spindle fibers, however, are not very distinct until late anaphase and telophase. In these stages the central spindle fibers extend the entire length of the spindle and often appear in groups. The spindle at this time is very broad in the equatorial

region and markedly barrel-shaped. Frequently the poles of the spindle converge towards bodies of deeply stained material, as shown in figure 17.

In late anaphase the chromosomes are generally well separated and easily distinguished (FIGS. 18, 47, 48), but in early telophase, with the appearance of the nuclear membrane, they begin to lose their individual identity. The dispireme stage is well marked in figure 19. The chromatic strands at first appear to be more or less even and homogeneous, but in later stages light regions appear in the threads, giving a vacuolated appearance. About the time of the appearance of the nuclear membranes around the daughter nuclei, large, round, red-staining bodies are visible in the nuclei, which have the appearance of nucleoles. In the upper of the two nuclei shown in figure 19, a single body is present. In figures 48 and 49, the nucleoles are shown after the dispireme has begun to break up. There is in these preparations no evidence of the aggregation of stainable material about the nucleole, so characteristic of the resting nuclei in figures 1–8. The dispireme disappears shortly, and the daughter nuclei pass into the condition shown in figures 48 and 49. Following this they take on the characteristic resting appearance. Scattered chromatic bodies appear at the stage shown in figure 50. Later an aggregated body of deeply stainable material appears (FIGS. 20, 21).

Cell plate and wall formation

The formation of the cell plate in *Nitella* and *Chara* begins late in the division stages, and the thickenings in the spindle fibers in most cases do not appear until the daughter nuclei are completely organized. These thickenings, in fixed and stained preparations, are formed in the equator of the spindle and appear to be swellings on the central spindle fibers. In many cells, the equatorial region of the spindle may be filled with irregular or round deeply stained granules (FIGS. 19, 27) of various shapes and sizes. These granules do not appear to be connected with cell plate formation, since, in my preparations very few of them are present in the equatorial region where and at the time the spindle fibers have begun to thicken. On the other hand, Zacharias (86) believes they are the cell plate elements, and Johow (31) claims that a row of these granules

is transformed directly into the cell plate. In figure 20, the thickening in the fibers is visible across the whole spindle. The process of thickening appears to be accompanied by a shortening of the fibers, as though the material of which they are composed were going into the cell plate. The plate becomes visible in the central region of the spindle first, and is apparently developed outward toward the periphery. In figure 21, the cell plate is visible in the center of the spindle but does not extend to the plasma membrane of the cell. While the spindle fibers are shortening, clear regions free from fibers of any kind appear between them and the daughter nuclei. In fixed preparations there appears to be no so-called granular "tropho-plasm" such as is described by Timberlake (79) for *Larix* in this region. With the growth of the cell plate toward the periphery and the shortening of the fibers, the daughter nuclei move nearer the equatorial region (FIG. 21) and often lie very close to the young cell plate.

The cell plate appears to grow from the central region toward the periphery of the spindle. At the time the plate appears in the central region, there are no visible thickenings in the peripheral fibers of the spindle. Figure 21 shows an early stage of cell plate formation in which it is present only in the center of the spindle and does not extend to the periphery of the spindle. The fibers beyond the ends of the cell plate are very numerous and dense and extend to the plasma membrane. It appears from this figure as though additional fibers have arisen in the cytoplasm at the periphery of the spindle to complete the growth of the cell plate, but whether this has occurred could not be determined with certainty from such preparations. Possibly these are the same fibers that appear to cross each other at the periphery of the spindle shown in figure 18. Figure 22 shows a later stage in which the cell plate has reached the plasma membrane. The successive stages in cell plate formation are more difficult to follow in cells of the antheridial filaments, due to their small size, but the process is the same here as in vegetative cells. In figure 49 two cells are shown in which the cell plate extends entirely across the broad spindle to the sides of the cell. Figure 50 shows a later stage where the plate is denser and thicker than in the preceding figure and appears to be fully formed.

Centrosomes

Owing to the fact that centrosomes have been described in various *Characeae* by Schottländer (70) and Kaiser (32), particular attention has been given to all kinds of granules and bodies lying near the nucleus and in the cytoplasm during each stage in mitosis, but no structures have been found that resemble or behave like the centrosomes of animal, algal, and fungus cells. In the resting condition, as well as in division, the nucleus is often surrounded by numerous granules of various sizes and shapes. Their size varies from that of a nucleole to a point where they are scarcely visible. Generally these granules or bodies stain like the nucleole and chromosomes.

These bodies often lie in contact with or near the nuclear membrane in a clear unstained region. In the resting cell they may be found in any region near or in contact with the nuclear membrane. In cases where they occupy such a position and lie in a clear space, they might easily be mistaken for centrosomes. Figure 1 shows a number of granules near and in contact with the nuclear membrane. In the cell here shown, two of the granules are approximately equal in size and surrounded by a clear space. They appear as two centrosomes, resulting from a recent division, which have begun to separate. In figure 11 may be seen two bodies similar in size near the nuclear membrane but further separated than those in figure 1. Figures 16a, 16b and 17 show cells in which a number of granules lie at or near each pole of the spindle. In figure 17, the poles of the spindle converge towards two large irregular bodies. This figure resembles those of Debski (10, *figs. 13, 14, 16, 25*) in which he shows a number of spindles whose poles are somewhat centered on deeply stained bodies.

It was doubtless the appearance of such granules near or in contact with the nuclear membrane that led Schottländer and Kaiser to claim the presence of centrosomes in the Characeae. The study of hundreds of cells has convinced the writer that these granules are of the same nature as the larger bodies lying scattered in the cytoplasm, and differing from the latter only in size and position. In no instances have astral rays or fibers been seen in connection with them.

Chromatic granules in the cytoplasm

No adequate description, it seems to me, has been given of the nature, origin, and distribution of the deeply staining granules and bodies of various sizes and shapes in the cytoplasm of Characeae. Kaiser (32) regards them as equivalent to Altmann's granules because of their staining reaction. Zimmermann (88), Migula (44), and Debski (11) believe they are extruded into the cytoplasm from the nucleus, and more specifically from the fragmenting nucleole. Götz (26) holds that the granules in the eggs are extruded from the egg nucleus shortly before fertilization. Riker (61) studied the granules in dividing cells of *Chara*, and believes that they originate from the nucleole, but are not included in the chromosomes and daughter nuclei when they are formed. He calls them prochondriosomes and holds that they first appear in the equatorial region of the spindle. From this position they migrate outward into the cytoplasm around the spindle and become chondriomes. Mirande (45) found corpuscle-like bodies in the cytoplasm of *Chara* which continue to multiply during mitosis and which have a staining reaction like nucleoli. He thinks that they have in part been extruded from the nucleus. In addition, Mirande describes chrondriomes near the nucleus which migrate to the periphery of the cell, develop central vacuoles, and become chloroplasts.

Mangenot (40) made an extensive study of the constituents of the cytoplasm in cells of *Chara fragilis* to determine the origin of the plastids and starch grains. He found that the cells are filled with long rod-shaped chondriosomes, and, in addition, around the periphery of the apical cell there is a continuous border of rounded and oval bodies each with a small region in the center, which gives the reaction of starch with iodine. In the young eggs, there is first a retrogression of the plastids until they are indistinguishable from the chondriosomes. The chondriosomes then elongate into chondrioconts and form a vesicle in the center, which stains like starch. These starch-containing chondrioconts rapidly increase in size until the whole egg appears filled with large starch grains, according to Mangenot. He describes a similar retrogression of the plastids in the antheridial cell, until they have completely disappeared.

A careful study was made of these chromatic granules to determine their origin, staining reaction and general distribution in successive mitoses, and their relation to the nucleus, chondriosomes, and plastids. Material fixed with mitochondrial fixatives was carefully compared and checked up with preparations killed in Flemming's, Merkel's, Bouin's, and chrom-acetic solutions. In *Nitella* and *Chara*, the chromatic granules are as numerous in the resting cells as in cells undergoing division. Figure 23 shows a resting cell of *N. gracilis* whose upper part, marked *x* in the figure, was almost filled with densely stained granules. The total volume of these granules in the cytoplasm appears to be almost as large as the volume of the entire nucleus. Strasburger (77, *fig. 3*) and Debski (10, *figs. 1, 2, 8, 9*) figure similar bodies in resting cells. However, they are inclined to think that the bodies are most numerous during mitosis, while Riker (61) believes they have their origin in the nuclear division stages, and does not figure them in resting cells. Some resting cells may be entirely free from granules, while other cells may have as many as a hundred densely stained bodies or granules. This condition is true of dividing cells as well. Figure 12 is a section of a cell in the late spireme stage in which there is but one granule in the cytoplasm. Figure 18 shows a cell in late anaphase with only a few granules present, while in figure 19 of a telophase, the upper nucleus is almost obscured by bodies of all sizes and shapes.

The distribution of the chromatic granules in cytokinesis, though irregular, is, nevertheless, characteristic. In apical cells and those that are to become the antheridia and oogonia, the resting nucleus frequently lies nearer the basal wall than the center of the cell, and the chromatic granules are generally more numerous in the upper part of the cell. They often lie in a somewhat semicircular group near, and sometimes in contact with the nuclear membrane, and in the cytoplasm of the upper part of the cell, as shown in figure 23. Accordingly, when the apical cell divides, it appears as if a greater number of granules is distributed to the upper of the two cells that result from the division. Very often the upper pole of the spindle of dividing apical cells is surrounded by numerous deeply stained bodies or granules. Generally the cell which is destined to form the node receives fewer granules from this division of the apical

cell; yet many young nodal cells have been found with as many as forty chromatic bodies in the cytoplasm. In the first four divisions of the antheridia the distribution of granules appears to be towards the external periphery of the cells. Figure 17 represents the horizontal division of an antheridium in which are shown numerous bodies scattered in the cytoplasm of the external peripheral part, marked *x*, of the cell; that is, the part of the cell nearest the periphery of the antheridium. In figure 19, a telophase of the horizontal division of an antheridium, approximately 30 bodies of various sizes are gathered about the upper of the daughter nuclei, while no granules are present in the lower part of the cell. Figures 16a and 16b show metaphases of the fourth division of an antheridium which divides the octants into inner and outer cells. Practically all of the granules lie at the outer pole, marked *x*, of the spindle, and it is likely that they will be included in the outer cell when division is complete. In the subsequent divisions of the antheridia, the chromatic bodies seem to disappear gradually. When the shield cells, manubria and capitula are well differentiated, and the cells of the antheridial filaments fully formed, very few and often no granules are to be seen in the cytoplasm of the latter cells.

The distribution of the chromatic granules in the cell which becomes the egg, after the so-called 'Wendungszellen' have been formed, appears very much the same as described in the apical cell. When the first 'Wendungszelle' is cut off tangentially from the egg cell, relatively few bodies are included in it. They are generally found in greater numbers in the egg, often distributed in the manner shown in figure 24. In some cases when the first 'Wendungszelle' is cut off apically, it generally receives more granules than when formed tangentially. This is not true of all cases, however. Very few chromatic bodies appear to be included in the second and third 'Wendungszellen' in *Nitella gracilis*. In figure 25 is shown an egg with the first and second 'Wendungszellen,' in which a large number of round and irregular bodies are present in the apical portion of the egg, while comparatively few are to be seen in the 'Wendungszellen.' When the egg is fully developed, the cytoplasm may often contain as many as 50 densely stained granules. Later, at the time of fertilization, a large number of granules is often aggregated near the apex of the egg.

The distribution of the chromatic bodies on the mitotic figure varies. They are frequently found extending in a row across the equatorial plate of the spindle, as illustrated in figures 19 and 27. In figure 19, granules are also present at the upper pole of the spindle as well. In figure 27, the deeply stained bodies on the spindle appear to be almost equal in volume to one of the groups of daughter chromosomes. The presence of granules in one place on the spindle does not exclude their presence at other places. At the same time that they appear on the spindle, they may be seen in great numbers in the cytoplasm. Often in early telophase, as shown in figure 19, the chromatic bodies may sometimes be so numerous around the poles of the spindles that the daughter groups of chromosomes are almost obscured. In figure 28 is shown an equatorial plate stage in which an unusually large number of granules and fragments are present at both poles of the spindle. Many cell division figures have been found in which the poles of the spindle converge towards a granule or a group of granules, as shown in figure 17. Where single granules lie in such positions, they may easily be mistaken for centrosomes. Many mitotic figures, on the other hand, may be found in which no granules whatever occur. Such a cell is shown in figure 18.

Concerning the size, shape and staining reaction of the chromatic granules, considerable variation has been found. Their size varies from that shown in figures 16a, 27 and 28 to a point at which they are scarcely visible. Some of the large, round granules are 3.5 microns in diameter, and I have found a few single bodies as much as 5 microns long and 2 microns in diameter. The bodies may be oblong, round or angular in shape; they may occur singly or in groups. In figure 23 may be seen several angular aggregated bodies in addition to the single ones, and it is apparent in this and many other similar preparations that such bodies are made up of smaller granules, so that they are irregular in outline. Their reaction to stains is generally the same as that of the nuceole. In Flemming's triple stain they may be deep red to faint violet; in haematoxylin, deep blue to black, and in aceto-carmine and saffranin, red.

A careful study of the granules with the mitochondrial fixative was made to determine their relationship to the chondriosomes described for *Chara* by Riker, Mangenot and Mirande.

Benda's, Němec's and Regaud's fixatives were used. The best results were secured with Benda's and Regaud's fluids. In cells fixed in these fluids and stained with haematoxylin and crystal violet-sulpharizarin, according to Benda's method, the chondriosomes are abundant, particularly near the nuclear membrane and the periphery of the cell. In addition to the chondriosomes in the cytoplasm of such cells may be found large round, oblong, and often angular-shaped bodies, which are different in size and shape from the chondriosomes. Such bodies are similar in every respect to the granules occurring in cells fixed in strong chrom-acetic solutions, Flemming's, Bouin's and Carnoy's fluids. Figure 26 shows a cell in which these granules are sharply contrasted with the chondriosomes. A comparison of this cell with those shown in figures 1, 17, 23 and 24, shows the similarity in size, shape, and general appearance of these granules in cells fixed in entirely different killing agents. There is no distinct border line of size in figure 26, however, between these granules and the chondriosomes. The smallest granules and the large chondriosomes are almost indistinguishable in size and shape, and it is impossible to differentiate the one from the other with certainty where they are so similar. The large granules, on the other hand, can easily be distinguished from the chondriosomes by size, shape, staining reaction, and general appearance. They appear to be much the same regardless of the kind of fixative used in killing the cells. Riker's contention that they are easily destroyed in strong fixing agents has not been confirmed from my preparations. In fixing fluids in which chondriosomes have been reported to be generally dissolved, the size, shape and distribution of the granules is essentially unaffected. They are as numerous in cells fixed in strong chrom-acetic solutions as in cells killed in fixatives lacking acetic acid. From these observations it appears to me that the large granules or bodies in the cytoplasm of cells of *Nitella* and *Chara* are distinctly different from the chondriosomes and should not be classified as such.

NUCLEAR AND CYTOPLASMIC CHANGES IN WATER AND SUGAR SOLUTIONS

I have tested quite fully the effect of aceto-carmine on the appearance of the so-called resting nucleus and the cytoplasm

in the antheridial filaments of *Nitella* and *Chara*. The stock solution of aceto-carmine, made up according to Belling's formula, was diluted with once-distilled water to the desired strength. A dilution of one part of aceto-carmine to eight parts of once-distilled water gave good results, and all of the cells shown in PLATE 13 were studied in this dilution. For comparison I have also studied and photographed these resting nuclei in tap and distilled water and in sugar solutions of various strengths. The cells of the antheridial filaments undergo a marked change in appearance within a few minutes after the filaments are teased out in the water, and similar, but less rapid, changes occur when they are mounted in sugar solutions.

These changes in the appearance of the nucleus and cytoplasm when the cells are mounted in water and sugar solutions take place with striking rapidity, as is shown in the figures in PLATE 12. As a means of presenting these changes as objectively as possible, I have photographed the cells at different specified intervals. These figures (PLATES 12, 13) illustrate the difficulties and weakness of photographic methods when used on cytological problems, but none the less, in view of our present interest in the mass appearances of protoplasm as bearing on the problem of its colloidal make-up, they serve as an excellent check and supplement to drawings of fixed and stained preparations.

Figures 52 to 57, and 58 to 61 are photographs of filaments mounted in tap water. The first series shows four nuclei in the same filament photographed at successive periods after mounting. In figure 52, photographed ten minutes after teasing out in water, the outlines of the nuclei are quite sharp as compared with nuclei when first mounted (FIG. 68). Several minutes are always required in mounting, locating, and photographing the filaments, and the appearance of the nucleus, in the meantime, undergoes a marked change. In the procedure which I have followed, from five to fifteen minutes elapse between the time when the antheridia are first broken open in the water and the time when the exposure is made.

The nuclei when first mounted appear comparatively homogeneous, but highly refractive, with very faint outlines. Figure 68 shows seven cells as little modified by treatment with water as any I have been able to secure in photographs. The conspicuous dark spots in this photograph are due to extraneous material

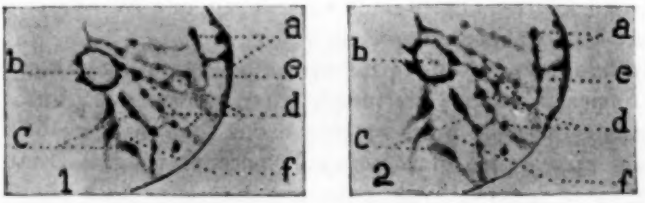
outside the filaments and are to be disregarded. In these cells very much the same appearance characterizes both the nucleus and cytoplasm, and little structural differentiation is visible. I regard the appearance of these cells as being very nearly that of the so-called resting nucleus unmodified by treatment, and for comparison I shall use these cells as the starting point in describing the nuclear and cytoplasmic changes in the series in PLATE 12.

Whether the nucleus in these cells is a two phase or polyphase colloidal system is not obvious from the photograph, but it is, nevertheless, evident that if colloidal phases are present in the nucleus, their indices of refraction are very much the same, and, consequently, show little visible differentiation. From the photograph the nucleus might be a molecularly dispersed solution of protein in nuclear sap, or a hyaline, optically almost homogeneous, emulsoid gel.

In figure 52, taken ten minutes after mounting in tap water, the nuclear outlines are much more conspicuous and their content is optically more heterogeneous than in figure 68. The general appearance of the nucleus perhaps suggests that it is made up of a more hyaline ground substance with the darker elements imbedded in it. There is, of course, no adequate evidence from such a figure that the clearer hyaline material is a watery nuclear sap in which dense chromatic elements are distributed, but the general appearance, none the less, suggests a confirmation of the conclusions as to the nuclear make-up which have been reached by a study of fixed and stained preparations. In the uppermost of the four nuclei shown in this figure, the dark elements appear to consist of rather rounded granules and more angular masses arranged in short strands and groups. With the view of bringing out more clearly what seems to me to be the appearance and arrangement of the light and dark elements in the series of photographs of the uppermost nucleus in figures 52 to 58, I have made a corresponding series of diagrams of a sector of this nucleus. In the region of the nucleus shown in text-figure 1, there are a number of short strands that appear to be made up of small irregularly-shaped bodies and granules. Next to the nuclear boundary is a nodular strand that extends a considerable distance around the nuclear periphery. Although more or less uniformly dark, its irregularity

suggests that it may be a connected series of bodies and granules. Parallel to it but slightly removed from the nuclear boundary is another perhaps similar strand which is likewise nodular in outline. These two strands designated by *a* in the text-figure appear to be connected in the center by a short, thin strand. The light region between these two strands is labelled *e* in the text-figure.

In a somewhat eccentric position in the nucleus may be seen a highly refractive rounded body, which is evidently the nucleole of fixed and stained preparations. Below the nucleole is the suggestion of two somewhat parallel, interrupted strands with an elongated light area, *f*, between them. These two strands, *c* in the text-figure, are not uniformly dark, and give the impression



TEXT-FIG. 1. Diagram of a portion of the uppermost nucleus of FIG. 52, PLATE 12. FIG. 2. Diagram of a portion of the uppermost nucleus of FIG. 53.

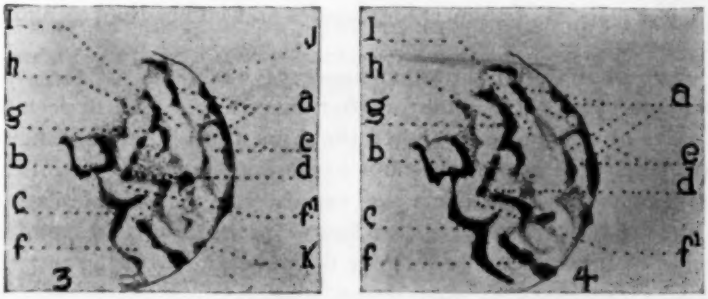
that they consist of more or less connected irregular granules. The relative light and dark appearance of these elements may be due to certain of them being more or less out of focus. There is a further suggestion of strands of connected granules in the region labeled *d* in the diagram. These strands appear to be somewhat radially placed around the nucleole.

As noted above, the general appearance of the nucleus suggests that the light hyaline areas make up a ground substance or continuum in which the dark elements are dispersed. The appearance is somewhat that of an emulsoid with the light elements constituting the medium of dispersion and the dark elements the dispersed aggregates. However, the presence of irregular strand-like elements made up of granules and bodies more or less connected suggests an interrupted reticulum suspended, perhaps, in an optically more homogeneous medium.

Figure 53 shows the same segment of the antheridial fila-

ment photographed five minutes later. Very little change is perceptible. The same elements are recognizable in the uppermost nucleus and in the same arrangement as they are shown in figure 52. The dark elements perhaps seem darker and denser. It is possible, however, that this may be due to a slight difference in exposure and development of the negative. Text-figure 2 is a diagram of this same nuclear region from figure 53. The same elements shown in text-figure 1 are recognizable throughout.

Figure 54 shows the same filament photographed twenty minutes after mounting. There is here a more marked change



TEXT-FIG. 3. Diagram of a portion of the uppermost nucleus of FIG. 54, PLATE 12. FIG. 4. Diagram of a portion of the uppermost nucleus of FIG. 55.

in the nuclear make-up than in figures 52 and 53. If we compare the appearance of the uppermost nucleus in this photograph with its appearance in figures 52 and 53, we note many differences in the nuclear elements and their arrangement. It is, of course, possible that these differences are due to a difference in the focal plane shown in figure 54. I have made a diagram of the corresponding sector of this nucleus for this figure, which is shown in text-figure 3. The black spot that is so conspicuous above and to the right of the nucleole is due to extraneous material outside of the nucleus and may be disregarded. The nucleole still appears as a highly refractive body, but its outline, due to the dark elements surrounding it, appears to be somewhat angular. Below the nucleole in the region occupied by the two strands labeled *c* in the two previous text-figures is a thick, dark strand-like element, *c* in text-figure 3, that can be traced from the nucleole to the periphery of the nucleus. Immediately

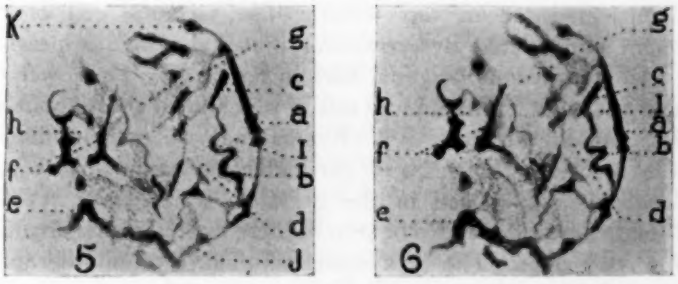
to the right of the nucleole is another dark element, *d*, somewhat zigzag in outline. Between these two strands a hyaline zone extends from the region labeled *h* through f^1 and *f* to the nuclear boundary. In the region marked *g* is a shorter, denser strand, and adjacent to the nuclear boundary, as in the two previous figures, is a dark irregular strand continuing from *j* to *k*. Somewhat parallel to it is another strand, *a*, and between them is an elongated hyaline area marked *e*. These two strands are very similar in position and appearance to the strands labeled *a* in text-figures 1 and 2. Between the elements *a* and *g* is another hyaline zone, marked *i*. In figure 54 the dark elements appear less like a series of connected granules and bodies than in figures 52 and 53.

The photograph shown in figure 55 was taken of the same filament twenty-five minutes after mounting. The uppermost nucleus of this figure has much the same appearance as in figure 54. The figure appears perhaps to show more contrast, and this again may be only a matter of variation in the photographing. I have, however, made a text-figure (4) of the corresponding sector of the uppermost nucleus in this figure to bring out the striking similarity in size, shape, and position of the nuclear elements as compared with those in the picture taken five minutes earlier.

Figure 56 shows a photograph of the same segment of the filament taken thirty minutes after mounting. In the uppermost nucleus very little is to be seen of the elements shown in figures 54 and 55. In this figure there is very little, if any, suggestion of granules or bodies arranged in series and groups, such as appear in figures 52 and 53. The nuclei are somewhat less in diameter, and the whole effect is somewhat that of being out of focus. The appearance of the nucleole, however, indicates that the focal plane cannot be very different, and figure 57 agrees well in general appearance with figure 56. Text-figure 5 is a diagram of the corresponding sector of the uppermost nucleus. Lying slightly over the highly refractive nucleole, *h*, may be seen two short, dense, branched bodies labeled *f*. Extending half-way around the periphery of the nucleus from *k* to *j* in text-figure 5 is a long irregular strand of varying density. In the portion marked *a*, it appears very dense and straight, and projecting downwards at an acute angle from this portion

of the peripheral strand is a short, thick element marked *c*. Below this is a conspicuous undulating strand labeled *b*. Between it and the peripheral strand is the elongated hyaline area, *i*. In the regions of the nucleus marked *g* and *d* are two conspicuous rather broad hyaline zones which may be continuous with each other. Below the region occupied by the nucleole is a long, dense, irregular strand marked *e*. In addition to these dense strands are other fine, lighter and less dense strands which appear to be connected with the denser elements.

Figure 57 shows the same filament photographed five minutes later, and the appearance of the nuclei in this figure is essentially the same as that shown in figure 56, except that the strand-like elements may be slightly darker and denser than in the preceding figure. Text-figure 6 shows the corresponding sector of



TEXT-FIG. 5. Diagram of a portion of the uppermost nucleus of FIG. 56, PLATE 12. FIG. 6. Diagram of a portion of the uppermost nucleus of FIG. 57.

the uppermost nucleus that was diagrammed in the previous figures. It is to be particularly noted in figures 56 and 57 that the nuclei are smaller than in the preceding figures. I have not observed swelling of the nuclei in water mounts, such as Schaede (68, *pl. 3*) describes for the root tip cells of *Allium Cepa*. In most cases of antheridial cells of *Nitella* and *Chara* which I have kept under observation, the nuclei appear to shrink in size rather than to swell. This is further shown in figures 60, 61, 66, 67, 70, 71, 79, and 80.

In figures 56 and 57, there is a marked change in the appearance of the cytoplasm as contrasted with its more or less optical homogeneity in figures 52, 53, 54 and 55. Dark and dense irregular strand-like elements differentiated from a hyaline

ground mass now appear in the cytoplasm. This change parallels that occurring earlier in the nuclei.

A comparison of figure 57 with figure 52 suggests that the visible modification of the nuclei as a result of being mounted in water consists in a change from a more granular to a strand-like appearance of the denser nuclear elements. If we begin with the stage first visible, as shown in figure 68, the suggestion that a more homogeneous sol has changed to a gel with the withdrawal of a large amount of water from the dispersed phase is to be considered. The appearance of strands and irregular masses in figure 57 suggests that water may have been withdrawn from the interstices of protein colloidal particles, bringing them into closer proximity as series of granules and finally strands.

In figures 58 to 60 is shown another series of figures of a filament mounted in tap water. The terminal cell of this filament is obscured by extraneous material, which appears as the two conspicuous diagonal bars in figure 58. The lower cell is also obscured. The middle cell is well shown throughout the series, and in this case it is obvious that we cannot identify in the successive figures any particular nuclear elements such as could be recognized in the previous series (FIGS. 52–57). No definite granules are to be seen in these figures, either arranged in rows or groups. The dark elements in figure 58, photographed fifteen minutes after mounting, are perhaps best described as flocculated masses more or less connected, but not obviously reticulated. It is not clear whether the differences in the two series are due to differences in the stage of nuclear development or differences in the effect of the medium. Figure 59 shows the same filament five minutes later. The particular elements visible in the central nucleus in figure 58 cannot be so easily recognized as such in figure 59, though a diagonal line of four bodies toward the top of the nucleus is perhaps recognizable in both figures.

The same filament is shown thirty minutes after mounting in figure 60. Similarly in this figure, no particular nuclear elements present in the previous figures are readily identifiable as in the preceding series at this stage. In figure 60 the central nucleus has a shrunken appearance, and the cytoplasm shows irregular, clumped masses. At the points marked *1* and *2*, are

large clear spaces that obviously suggest vacuoles. In figure 61, taken thirty-five minutes after mounting, the nuclei and the filament in general show a tendency to collapse.

The effect of once-distilled water on the appearance of the nucleus and cytoplasm is the same as that of tap water. Figures 62 to 67 show a further series of photographs of a filament mounted in once-distilled water made at intervals of five minutes. The nucleus marked *a* is perhaps typical for the series. Although the nucleus is very faintly differentiated in figure 62, the nuclear boundary is fairly sharp. The nuclear content at this time is still quite homogeneous, but with some suggestion of granules. However, the granules are by no means as clearly visible as in the first photograph (FIG. 52) of the tap water series. We can also recognize some dense, short strand or shred-like elements lying in the hyaline ground substance. There is already without doubt the suggestion in this figure of microscopically visible colloidal elements, granules, strands, and flocculated masses dispersed in a more hyaline continuum. Figure 63 shows the same nucleus five minutes later. As in the case of figures 52 and 53 we can identify in this figure particular light and dark elements that are recognizable in the preceding figure. These particular elements, very faint in figure 62, appear denser and darker and somewhat sharper in outline in figure 63. In figure 64, on the other hand, a marked change in the appearance of the nucleus is visible, and none of the particular elements that are present in figures 62 and 63 can be recognized in this figure. This is, at least, in part a matter of the photographing. The whole filament is darker and denser in appearance. Granules and strands are recognizable in the nucleus *a* as in the preceding figures, and these elements appear denser and larger. This increase in size and density of the dark elements suggests that the degree of dispersion of the discrete elements has perhaps decreased.

Figure 65, taken five minutes later, is strikingly like figure 64, and we can identify many corresponding elements in both figures. Figure 66, photographed thirty minutes after mounting, shows the same change noted at a corresponding stage in the preceding series. The nuclear elements consist of large, irregular, dense strands and flocculated masses that are not recognizable in figure 65. Moreover, the nucleus appears to have decreased

considerably in volume. The cytoplasm also shows dense clumps and flocculated masses, and does not have the same optical homogeneity that characterizes the previous figures.

In figure 67, taken thirty-five minutes after mounting, the changes noted in figure 66 are perhaps still more intensified. No particular strands, granules or flocculated masses that are present in the preceding figures are identifiable. The nucleus *a* is smaller than in figure 66. This series suggests that the effect of distilled water on the appearance of the nucleus and cytoplasm is very similar to that of tap water.

Figures 68 to 71 show a series of photographs made at different intervals of an antheridial filament mounted in one and one-half per cent cane sugar solution, made up with once-distilled water. Figure 68, photographed five minutes after mounting, comes the nearest to representing the appearance of a typical so-called resting nucleus when first mounted and before it has become affected in any way by the mounting medium. The nuclei marked *a* and *b* may be taken as typical of the filament. In figure 68, in contrast to figures 52 and 62 of the tap and distilled water series, very little except a faint nuclear boundary is to be seen, and, as noted before, the nucleus as a whole appears comparatively homogeneous. Figure 69, photographed thirty minutes after mounting, shows the differentiated elements recognized in the previous series. The nuclear boundary is sharp, and the nuclei have perhaps a more uniformly coarse granular appearance. The general appearance of the nuclei suggests that the hyaline areas represent a continuum in which the darker elements are dispersed.

In figure 70, the same filament photographed one hour after mounting, we see even more conspicuously than in the previous series the effect of long immersion in the sugar solution. The coarsely granular appearance of the nuclei has apparently disappeared, and in its place we find dense coarse strands and flocculated masses with irregular light areas between. The appearance of the cytoplasm has likewise undergone a decided change, with the appearance of strands and flocculated masses. The nuclei marked *a* and *b* in this figure do not occupy the central position in the cell as in figures 68 and 69, but appear to lie displaced against the cell wall. This condition is even more conspicuous in figure 71 of the same filament, taken two hours

after mounting. The nuclei marked *a* and *b* in this figure, as well as in figure 70, are much smaller than in figures 68 and 69, and appear somewhat shrunken. In one corner of each of these two cells we see large light areas that suggest vacuoles. Moreover, the nuclear boundaries in figures 70 and 71 are not as sharp as in the preceding figures, and there appears to be a layer of flocculated cytoplasm adhering to the nuclei.

The effect of a three per cent cane sugar solution on the appearance of the nucleus and cytoplasm is shown in figures 72 and 76. The successive changes in appearance in this series are much the same as in the water and one and one-half per cent cane sugar solutions, with the exception that apparently the changes take place more slowly than in the other media. As in figure 68 of the previous series, very little except a faint nuclear boundary is to be seen in the apical cell of figure 72, photographed five minutes after mounting, but in figure 76, two hours later, its nucleus has much the same appearance as the nuclei in figures 54, 55, 65, and 66. Filaments mounted in three per cent cane sugar solutions usually begin to swell after three or four hours, but swelling is not yet visible in figure 76.

Figures 77 to 80 show a series of photographs of a filament mounted in a five per cent cane sugar solution. Figure 77 shows four cells photographed five minutes after mounting, and in figure 80 we see the same cells six hours later. The changes in the appearance of the nuclei are of the same type as shown in the previous series. In figure 80 the cells are shown after six hours in the sugar solution, three times as long as was the case for the filament shown in figure 76. It is obvious in this figure that plasmolysis has occurred. In the regions marked *a* and *b*, the cytoplasm has obviously drawn away from the cell wall.

Figures 81 and 82, 83 and 84 show the effect of aceto-carmine, following a certain period in cane sugar solution, on the appearance of the nuclei. Figure 81 shows four terminal cells of a filament photographed five minutes after mounting in a three per cent cane sugar solution. The large conspicuous light and dark spots are due to extraneous material and are to be disregarded. The nuclear boundary in the second cell from the apex of the filament is sharp and clear, and the nucleus as a whole has a granular appearance with some suggestion of short strands. However, the light and dark elements are not clearly

differentiated, and, as in figure 68, the nucleus is comparatively homogeneous. The same nucleus is shown in figure 82 after being mounted five minutes in aceto-carmine. Ten minutes elapsed in making the change from the sugar solution to aceto-carmine. Considering the rapidity with which the appearance of the nucleus changes in sugar solutions, as is shown in figures 52 to 80, it is probable that the change in the nuclei shown in figure 82 may be due in part to the effect of the sugar solution. Nevertheless, the change is very marked. In figure 81, the second nucleus from the apex of the filament has a somewhat granular appearance; in figure 82, it shows many dense irregular fragments, short strands, and flocculated masses of material. None of these dense elements can be seen in figure 81, and they are apparently due to the action of the sugar solution and aceto-carmine. The nuclei in figure 82 are very similar in appearance to those in figures 54 and 55. A comparison of the second nucleus from the apex of the filament as it appears in figures 81 and 82 gives the impression that the aceto-carmine has brought about an aggregation of the denser elements into larger masses or aggregations, leaving conspicuous light areas between them.

Figure 83 shows three cells of another filament photographed after ten minutes in a three per cent cane sugar solution. Unlike the second nucleus from the apex in figure 81, the central nucleus in this figure already shows conspicuously differentiated elements. The nuclear boundary is very sharp, and within it the nuclear material gives more or less the suggestion of a reticulum. The filament shown in figure 83 was in the sugar solution five minutes longer before photographing than that of figure 81, and the difference in appearance between the two may in part at least be due to the effect of the longer exposure to the sugar solution. Figure 84 shows the same filament photographed five minutes later after being mounted in aceto-carmine. The effect of the aceto-carmine on the appearance of the nucleus is not as marked as in the case of figure 82, but is similar in its general character. It is to be noted particularly in figure 84 that we can recognize several of the same particular light and dark elements in the nucleus that are shown in figure 83. It appears from figure 84 that the aceto-carmine has not notably altered the appearance of the light and dark elements, but I regard figure 82 as showing

more typically the effect of aceto-carmine on the appearance of the nucleus.

In figures 85 to 90 is shown a series of photographs of four terminal cells of an antheridial filament mounted in aceto-carmine, made at intervals of several hours over a period of thirty hours. Figure 85 is a photograph made five minutes after mounting; figure 89 shows the same cells twelve hours later. Allowing for slight changes in appearance that may be the result of difference in length of exposure, development and printing of the negatives, these two figures are essentially alike as far as the appearance of the nuclei and cytoplasm is concerned. In figure 87, photographed three hours after mounting, there is no apparent change in the appearance of the nuclei, but the bulging of the cells between the cross walls suggests that they have begun to swell. This bulging becomes more marked in figure 88, photographed six hours after mounting. Figure 90 shows the four cells thirty hours after mounting. In this figure the outlines of the nuclear elements are not so sharp and clear as in the previous figures, and the filament as a whole appears much darker. This change is characteristic in aceto-carmine mounts kept for a considerable length of time. After twelve to eighteen hours the cytoplasm becomes rather diffusely stained with the carmine. The bulging of the cells, conspicuous in figures 87, 88 and 89, has apparently disappeared in figure 90. However, it is common in the aceto-carmine mounts for the cells to continue to swell until they burst.

Careful observation of preparations such as are shown in figures 81 and 82, 83 and 84, and 85 to 90, indicate that the changes that may result from treatment with aceto-carmine take place very quickly and that after these initial changes there is little essential modification in the appearance of the nuclei for several hours after mounting. In general the visible effect of aceto-carmine, made up according to Belling's formula and diluted in the manner previously described, on the appearance of the so-called resting nucleus in the antheridial filaments of *Nitella* and *Chara*, is to bring out the nuclear boundary more clearly and to cause what appears to be an aggregation of the denser nuclear elements visible in unstained and untreated nuclei into larger and more clearly differentiated irregular dark shreds, bodies, and strand-like aggregations.

The nuclear elements in such preparations as are shown in PLATE 12 are of course difficult to study and interpret, but in view of the many uncertainties as to the nature and behavior of the so-called chromatin granules, nucleole, nuclear sap, etc., it is certainly worth while to give renewed attention to their appearance in the living condition or as slightly modified by fixation as possible. These photographs illustrate the weaknesses of microphotography for showing cytological data, but they present at least an objective view of the mass changes that result from treatment of living cells with water, sugar and aceto-carmine solutions.

NUCLEAR AND CELL DIVISION IN ACETO-CARMINE PREPARATIONS

Figure 91 shows an aceto-carmine preparation photographed one hour after mounting, in which the nuclei stand out more conspicuously against the cytoplasm and appear more dense and opaque than in figure 68. This difference is probably due to the effect of aceto-carmine, as is shown in figures 81 and 82. Within the nuclei of figure 91 may be seen light and dark elements as in the figures in PLATE 12. In none of these nuclei in figure 91 can be seen the compound nucleole-like mass of material that is so conspicuous in the fixed and stained preparations shown in PLATE 12.

I have arranged as a series in figures 92 to 100, photographs showing the early prophase changes of the nucleus in aceto-carmine preparations. The seriation offers very great difficulties and the arrangement of the figures is merely tentative. The later stages of division following the equatorial plate are, of course, much more easily recognizable and their seriation is an easy matter, as Strasburger (74, 75) early showed in his studies on stamen hair cells of *Tradescantia*. I am presenting what I consider early prophase stages merely for comparison with the later stages after the chromosomes are fully differentiated, and regard the seriation presented as preliminary and requiring further study.

Figure 92 is from a photograph of a filament taken half an hour after mounting. The nuclei in this preparation appear less dense than in figure 91, but it is not certain that the stage shown here may not precede the stage shown in figure 91. In figure 93, photographed one hour after mounting, there is

perhaps the suggestion of a coarse reticulum. The light material between the dark elements also seems very clearly to be a continuum in which the dark elements lie. The appearance of strands and perhaps a reticulum is seen also in the apical nucleus of figure 94, photographed thirty minutes after mounting. The strands that apparently make up the reticulum appear comparatively thick and dense in certain regions and very faint and thin in other portions. This difference, however, may be due to certain portions of the reticulum lying out of focus.

What is perhaps a later stage than the one shown in figure 93 is seen in figure 95, photographed forty-five minutes after mounting. However, the suggestion of a reticulum of dark masses connected by thin, lighter strands is not as obvious as in the two preceding figures. The dense material is more uniformly dark, and the light material here also appears to form a continuum.

Figure 96, photographed two hours after mounting, certainly shows a much later stage than those shown in the preceding figures. The dense black elements in the nucleus of the terminal cell are considerably smaller in number but much larger in size than those shown earlier. There are approximately eleven large, intensely black, variously shaped bodies to be seen in this nucleus. Near the center of the nucleus are three large dense bodies, angular and rounded in outline and connected by thin strands of almost equal density. In the right hand portion of the nucleus is a conspicuous element with two hyaline spots in it, and there are other similar dense elements with pale areas in them. It is possible that in some cases these light areas may be high light spots of globular, dark elements. The dark bodies are visibly connected by longer or shorter thin dense strands. Similarly the hyaline areas between the dark elements are larger in size but comparatively smaller in number than in any of the preceding figures. The jagged and angular contour of the dark elements, so evident in figure 95, has disappeared to some degree in figure 96, and although still somewhat angular, these elements appear more rounded and elongated.

Figure 97 shows the apical cell of a filament photographed an hour and a half after mounting, in whose nucleus the suggestion of thick bands of dense material is very obvious. The dense elements in this nucleus and in the following stages are

sufficiently definite and small in number to be counted with some degree of certainty, and they, doubtless, are the chromosomes of fixed and stained preparations. There are approximately nine dark, conspicuous elements, numbered consecutively, in this nucleus. The presence of thin connecting strands between these bodies is not as evident as in the previous figure, and the dark elements are not as irregular and angular. Futhermore, in figure 97 the dense, thick band- or even ribbon-like elements give the impression of being somewhat curved and twisted. This is suggested particularly in the case of the elements numbered *1*, *2* and *6*. The appearance of the less dense material suggests that it is a part of the bands lying somewhat below the focal plane.

In figure 98, taken one hour after mounting, the short bands or ribbon-like elements appear sharper in outline than in the previous figure, and the hyaline areas between are likewise more clear and sharp. We can count in this photograph approximately seven dense, black elements which still appear somewhat connected. Element number *2* is obviously connected by a thin dense strand with number *1*, and similarly the elements numbered *3*, *4* and *5* appear connected. The hyaline area numbered *9* has the shape of a wide U, and extends downward between elements *7* and *2*, forming a light zone that resembles a distorted Y. Number *8* is another long hyaline area extending from element number *5* to the thin connecting strand between elements *1* and *2*. No nuclear boundary is visible around the upper part of the nucleus, but around the lower half may be seen a fine line which suggests the so-called nuclear membrane.

Figure 99, photographed two hours after mounting, is perhaps a later stage than that shown in the previous figure. In the lower right hand portion of this nucleus may be seen a thick dense band that extends half way around the periphery of the nucleus from the point marked *4* to *9* on the other side. In the center of the nucleus lies another band, numbered *3*, that appears somewhat coiled and twisted, and seems to disappear below the focal plane at *1*. At *5* is a straight band somewhat club-shaped at the upper end, and the element marked *8* and *6* appears to be a single mass of material whose central portion perhaps lies below the focal plane. The dense rounded element at *7* is connected with element number *3* by a thin dense strand.

The suggestion is very obvious in this nucleus that the hyaline zones constitute an optically structureless continuum in which the band- and ribbon-like elements lie.

In the central cell of figure 100, photographed one hour after mounting, we get the impresson of a somewhat rounded group, with a tendency for the elements to be parallel and concentric. Extending from the region marked *1* to *9*, the elements appear somewhat continuous. In the region labeled *2* is a curved, rather pale band which connects with the same band at *3*. At *4* and *10*, we get the suggestion of strands which are rather pale. The dense element marked *6* appears to be a short portion of a ribbon, and at *7* and *8*, we have two other dense irregular bands. Figure 100 is more aberrant in its more generally hyaline and homogeneous appearance. Figures 91 to 100 show certain changes which occur in the prophase stages of the nucleus in antheridial filament cells of *Nitella* and *Chara*. Their interpretation is difficult and the seriation given, as noted, is tentative.

Figures 101 to 103 may represent the same stages as seen in slightly higher magnification. In the central nucleus of figure 101, I have numbered consecutively three thick bands extending diagonally across the center of the cell. At *5* is a short thick element. In figure 102 the nuclear boundary seems to be still present. In this cell, photographed two hours after mounting, the dense elements still appear more or less connected. At the upper pole of the nucleus is a conspicuous band, marked *7*, and extending from *1* to *5* is an interrupted element, parts of which seem to lie below the focal plane and give the impression of being curved and twisted. At the points marked *1*, *2* and *5*, it is very dense. Across the center of the nucleus is a thick dense mass of material, which appears to extend with varying thickness and density from *9* to *6*. At *4* it appears very dense and thick, and at its lower end it appears to be connected with the element labeled *3*. At *8* and *3* are two other dense bands. In figure 103, two large chromosome-like elements are conspicuous at *5* and *6*, but the other dense elements numbered consecutively in this nucleus are not as sharp and distinctly separated as these. In this nucleus we still get the suggestion of a bounding membrane around the nuclear elements.

If we compare the nuclei in the successive figures from 91 to 103, we are impressed with the apparent increase in size but

decrease in number of the dense elements as the prophase changes progress, until in figure 97 we can begin to count them. In figures 99 to 103, a relatively small number of very large, dense elements appear lying in a more or less optically homogeneous ground substance.

I have not seen in living cells and aceto-carmine preparations definite spindle fibers such as are present in fixed and stained cells, except perhaps in figures 107 and 108. No polar caps or felted zones are recognizable in any of my preparations, nor could distinct fibers be seen in equatorial plate, anaphase, and telophase stages. In a number of cells in late anaphase and telophase stages, however, I have seen faint thread-like elements in the cytoplasm between the chromosomes and daughter nuclei that suggest the presence of spindle fibers. In figures 107 and 108 we see evidence of spindle fibers. Figure 108 shows a late anaphase or early telophase of two cells in which distinct fiber-like and cell plate elements are visible between the daughter nuclei. This filament had been mounted in tap water twenty minutes previous to photographing, and I have not so far obtained further figures confirming the appearances shown in it. Figure 107, an aceto-carmine preparation, shows four terminal cells of a filament in which fiber-like elements are visible between the anaphase groups of chromosomes. However, the basal end of this filament had been injured. It is to be further noted that in none of the other aceto-carmine preparations shown in PLATE 13 are spindle fibers visible.

Strasburger (74, 75), Behrens (3), Lundström (38), Samassa (65), Zacharias (87), Fischer (21) and Sands (66) failed to find evident spindle fibers in living cells of *Tradescantia*. Demoor (12), De Wildeman (13) and Schaede (68), on the other hand, figure them in abundance in this genus. In *Spirogyra*, Strasburger (75) describes the appearance of spindle fibers in living cells. De Wildeman has also figured them for *Spirogyra*, but his observations are contradicted by Behrens. In a few cases in *Epipactis* and *Orchis*, Treub (81) describes spindle fibers in living cells, but Zacharias fails to report such structure in living cells of *Larix*, *Cucurbita* and *Helleborus*. The later investigations of Lundegårdh (37) on *Allium*, *Vicia* and *Cucurbita*, and of Chambers and Sands (8) on *Tradescantia* indicate that spindle fibers are not visible in living plant cells. Schaede (68, pp. 240–

241), however, in a later study than Lundegårdh's, maintains that spindle fibers are plainly visible in living cells of *Allium*, and Showalter (72, *fig. 3*) figures spindle fibers in living germinating spores of *Pellia*.

Figures 105 to 115 show stages which appear to correspond to the so-called equatorial plate, metaphase, anaphase, and telophase stages of fixed and stained preparations. Figure 105, photographed one hour after mounting, shows four cells, in one of which, *A*, the chromosomes are in the equatorial plate stage. The large size of the chromosomes of this species of *Nitella* is very evident in this cell. The long chromosome *1* extends from the equatorial region at *4* to almost the end of the cell at *1*. The chromosomes marked *2* and *3*, as well as the two labeled *1* in cell *B*, show a light central line throughout their length, but it is not obvious here whether this light median line is evidence of a longitudinal split or whether it is a high light effect of the refractive chromosomes when slightly out of focus. A somewhat similar light central line is visible in the late anaphase chromosome, marked *1*, in figure 109.

Figure 106 shows two cells, photographed two hours after mounting, in which the chromosome groups are in a late anaphase or diaster stage. The chromosomes of *Chara sp.* are so numerous and close together that it is difficult to bring them out individually in photographs. In the terminal cell of this filament, however, we can see a thick, U-shaped chromosome, marked *1*. It is to be noted that the daughter groups of chromosomes in the lower cell lie almost parallel to the long axis of the filament, while in the upper cell they are diagonally placed. It is obvious from such a figure that if the cross wall, which is to be later formed between the daughter nuclei, is to be transverse to the filament, the whole division figure will have to rotate approximately 90 degrees. A later stage is shown in figure 109, taken an hour after mounting, in which the chromosomes have reached the ends of the cells. Figures 109, 110, and possibly 111, resemble the diaster stages of fixed and stained preparations.

In figure 109 the outlines of a few of the chromosomes are visible, and although not individually differentiated in figure 110, the orientation of the groups of chromosomes as a whole suggests that of a diaster. The position of the groups of chromosomes in the cells of these figures is noteworthy. In figure 110, most

of the daughter groups of chromosomes lie in diagonally opposite corners of the cell. This is even more pronounced in figure 111. In nine cells of this filament the groups of chromosomes lie in diagonally opposite corners. Moreover, in the cells between *1* and *2*, the long axis of the division figures in adjacent cells is not parallel, and lines drawn so as to pass through the long axes of all of these division figures would be more or less regularly zigzag.

Figure 111 appears to show a later stage than figure 110. In this stage the chromosomes are closely massed together, and it is impossible to identify them individually. In some of these cells the chromosome groups are triangular in shape, conforming to the corners of the cells in which they lie. This is particularly noticeable in the cells marked *2* and *3*.

Figure 112 probably represents an early telophase. The appearance of the daughter nuclei in these two cells gives the impression that they are in the so-called dispireme stage of fixed and stained preparations. The nuclei are flattened in shape and lie in the ends of the cells, but it is not very clear whether nuclear boundaries are present. Near the center of the nucleus marked *x*, lies a dense dark body, labeled *1*, which suggests a nucleole. However, it does not have the characteristic appearance of the nucleoles shown in figures 52 and 53. Around the periphery of the nucleus between the points marked *2* and *3*, the dark elements appear continuous, but this may be due to overlapping. At the regions marked *3* and *5*, this continuous dark element appears to be connected with the dense element labeled *4*. There are large, conspicuous hyaline areas in this nucleus; one in the center in which the nucleole-like body lies, and one on each side of the central area. As in the prophase nuclei, these hyaline areas appear to constitute an optically homogeneous ground substance in which the dense elements lie.

What is perhaps a still later stage is shown in figure 113, photographed one hour after mounting. The nuclei in this figure are more uniformly dark, and the dense elements consist of irregular strands and angular bodies. In the terminal nucleus of this filament, however, we can still see some evidence of longer, irregular band-like elements. Four such strands are numbered consecutively in this nucleus. On the right of the nucleus is a long dense band, marked *1*, and more or less parallel

to it are three other irregular strands, apparently connected at places by lighter, thin elements.

In figure 115, the nuclei have a more rounded outline, and the suggestion of long strands and dispireme threads has disappeared. However, in the regions of the apical nucleus of this filament, which I have indicated by numerals, we see suggestions of strands that are slightly denser and darker than the other elements in the nucleus. The general appearance of the nucleus is very similar to that of resting nuclei.

The changes that have apparently occurred in the appearance of the nuclei in the figures from 111 to 115 give the impression that the large, dense elements—chromosomes—of the late anaphase break up into smaller and smaller aggregates as the telophases progress, until a state is reached where the daughter nuclei have the appearance of resting nuclei. The dense elements appear to become more highly dispersed.

The process of cell plate formation is difficult to see in living cells and aceto-carmine preparations. As noted above, I have not seen definite spindle fibers in living and aceto-carmine treated material, except perhaps a few traces of such structures in late telophase stages. In figure 108, as previously noted, definite fiber-like elements are visible between the daughter nuclei, and across the equator of what look like spindles in these cells may be seen a faint lighter zone, whose appearance suggests an early cell plate. In figure 114, the cell plate appears as a faint line between the telophase nuclei, and in figure 115, it is fully formed and in contact with the horizontal walls of the cell.

The observations presented from fixed and stained preparations, together with the appearances shown in these aceto-carmine preparations, are strong evidence that cytokinesis in *Nitella* and *Chara* takes place by the progressive growth and development of a definite cell plate, which is later followed by a cell wall in the same manner as in the higher plants.

DISCUSSION

The changes occurring in the appearance of the nuclei and cytoplasm of antheridial filament cells of *Nitella* and *Chara* when mounted in water and sugar solutions are fairly definite and constant. When so treated, the outlines of the nuclei be-

come sharper and more distinct and their contents coarser, more granular and reticulum-like. Nägeli (51, *fig. 11b*) reported an injurious effect of water on the antheridial filament cells of *Nitella flexilis* as early as 1844, and both Nägeli (50) and Von Mohl (47) described contraction of the cellular content of pollen and spore mother cells as a result of being mounted in water. A few years later, Hofmeister (28, p. 428; 29, *fig. 2*, p. 6) found that pollen mother cells became quickly disorganized after being mounted in water. In some instances, he reports that so-called coagulation took place before the cells could be brought into focus. In 1880, Baranetzky (1) and Strasburger (74, p. 146–147; also 76, p. 299) report an increased clearness and sharpness in outline of the nuclear elements as a result of their being mounted in water. Lundegårdh does not specifically recognize any change in appearance of the nucleus and cytoplasm of root tip cells of *Allium Cepa* and *Vicia Faba* as a result of treatment with water and sugar solutions, but he reports that the structure of the nucleus is better differentiated in a preparation one-half to one hour old than in one freshly mounted.

These changes in the appearance of the nucleus as a result of being mounted in water and sugar solutions, as shown in PLATE 12 of my preparations, suggest possibly a closer and closer approach of colloidal particles or aggregates by withdrawal of water from the dispersed phase, until microscopically visible, irregular granules, bodies, and strands, such as are shown in figures 56, 57, 59, 60, 61, 66, 67, 70, 71, 79 and 80 are formed. The gradual emergence of conspicuous vacuoles in the cells as the appearance of the nucleus changes is perhaps the visible expression of similar changes in the cytoplasm.

As is shown in figure 68 of my preparations, and by Schaede (68, *pl. 3, figs. 1a, 3, 4*), differentiated nuclear elements are not microscopically visible in the cells when they are first mounted. This is not, however, adequate ground, it seems to me, for assuming that the resting nucleus is not colloidal. The indices of refraction of the different phases may be so much the same that little structural differentiation is visible. On the other hand, it is possible that the hydrolytic dispersion of the dispersed phase is so great that no microscopically visible elements are present when the cells are first mounted. It is none the less obvious in the different series shown in PLATE 12 that the apparent decrease in

the degree of dispersion of the denser elements as the changes continue is paralleled by an apparent increase in their size. These changes, it seems to me, appear to be similar in many respects to the changes that occur in the aging of dilute starch, described by Ostwald and Fischer (56). According to these authors, there occurs in the aging of dilute starch not only a decrease in dispersion but also a dehydration of the dispersed phase. The colloid particles not only appear to give off a part of their water, but they clump together to form larger aggregates. In the figures shown in PLATE 12, it appears as if the water that is perhaps withdrawn from the interstices of the dispersed colloidal protein phase accumulates in certain regions of the cell, forming large vacuoles, such as are shown in the cells marked *1* and *2* in figure 60, and *a* and *b* in figures 70 and 71.

The assumption that these changes in the appearance of the nucleus and cytoplasm are indicative of injury or death, and that the dispersed protein particles have aggregated together into microscopically visible particles by withdrawal of water from their interstices, is in line with the well known fact that protoplasm which has been injured in any manner, by frost, for example, as described by Nägeli (52) and Molisch (48), or by heat and chemical agents, is more permeable. It allows the cell sap, which in living and growing cells is always subject to high pressure, to diffuse out as if it had become porous. This is well shown by the familiar fact that when cells with coloured sap are frozen or heated to high temperatures, the pigment diffuses out readily, though this does not occur as long as the cells are living. Pfeffer's (59) extensive observations, duplicated in part by later studies by Fischel (20), Overton (58), Ruhland (64), Meyer (42) and Schaede (67), on the absorption of aniline dyes by protoplasm, indicate further an increased permeability of the cell after death. Pfeffer found that living protoplasm is impermeable to aniline dyes, and only after it is injured or dead are these stains permitted to penetrate the plasma membrane and other parts of the cell.

That the earlier changes in appearance of the cells shown in PLATE 12 are indicative of death or the approach of death, is not to be regarded as proved. They are, none the less, very similar to the so-called disorganization appearances accompanying death described by Strasburger (74), Klemm (35) and Schaede

(68). Strasburger reports that when pollen mother cells of *Tradescantia* are first mounted in well water, nothing is to be seen in the nuclei, but very quickly as the water begins to affect the cells, the nuclear elements become coarser and more clearly differentiated. This change continues, as death approaches, until the nucleus and cytoplasm are completely disorganized. Klemm found that treatment of the protoplasm of various plants with intense light, high and low temperatures, acids, alkalis, and other substances, produced marked changes in appearance as the cells died. The disorganization appearances, as he reports them, were extremely varied and differed according to the rate of death, whether slow or rapid. In general, Klemm describes the changes as granulation, vacuolization, coagulation and precipitation. He reports that as the protoplasm dies, it may take on a fibrillar appearance or become granular or vacuolated; the granules often uniting to form chains and net-like structures. Although Hofmeister (29) claims that contraction is a general and consistent accompaniment of death, Klemm found that this is not universal, even when there is an immediate loss of turgor. He reports the changes in the appearance of the nucleus to be the same as in the cytoplasm. In the root tip cells of *Allium Cepa* and *Vicia Faba*, Schaede (68) finds that the nucleus is optically homogeneous when first mounted in water and sugar solutions, but after the elapse of one-half to one hour, it becomes swollen and its content coarsely granular. He interprets this change in the nucleus as indicative of the gradual death of the cell. Similar modifications in the appearance of the nucleus are reported by Miehe (43, *fig. 8*) and Nestler (54) for cells in the vicinity of other mechanically injured cells.

Pfeffer (60) regards absorption of dyes, non-plasmolyzability, and permeability of the protoplasm to soluble pigments in the cell sap as indicative of death. Bechold (2, p. 302) states that with the occurrence of death, protoplasm gelatinizes and appears ultramicroscopically as a conglomeration of reflecting platelets. If the protoplasm is killed quickly, stiffening occurs; if it dies slowly, flocculation takes place. It is to be noted in connection with Bechold's observations, that in figures 58, 59 and 60 of my preparations, the central nucleus has a highly flocculated appearance. Chambers (7) notes that in the death changes the cytoplasm "may suddenly set, forming a rigid coagulated

mass. The coagulation structure gradually coarsens with the production of a network or granular precipitate."

It is to be particularly noted in the series of figures shown in PLATE 12 that the nuclei are apparently the first parts of the cell to change in optical appearance. Schaede (68) likewise emphasizes this for the root tip cells of *Allium* and *Vicia*. It is only after the elapse of several minutes that a marked change is visible in the appearance of the cytoplasm. It was particularly noted by Klemm (35) and Pfeffer (60) that the different parts of the cell do not all have the same powers of resistance to poisons. Pfeffer (59) found that the nucleus would often take up a stain while the cytoplasm remained entirely unstained; and it often happened that a poison killed the nucleus before the cytoplasm was fatally injured. Klemm reports that the nucleus is poor in resistance to electric shock, and that the nucleus can be killed while the cytoplasm remains alive for several hours. In a similar manner, it may be that the tap and distilled water change the appearance of the nucleus before the cytoplasm is affected.

The change in position, shape and appearance of the nuclei as a result of mechanical injury to the antheridial filaments is noteworthy. As previously described for figure 51, not only is the appearance of the nuclei in the immediate vicinity of the injury affected, but in less degree that of nuclei far removed. As noted before, I have observed the effects of mechanical injury on the position, shape and appearance of nuclei thirty cells removed from an injured cell. According to the observations of Tangl, Nestler, Miehe, Schürhoff, and Němec, this wound reaction of the protoplasm of neighboring cells is general in the plant kingdom. The reaction may even involve the passage of the nuclei through the cell wall nearest the injury (Miehe 43, *figs. 2, 3, 4*). Nestler reports that the effect of mechanical injury to a cell in the epidermis of *Tradescantia viridis* could be detected by changes in the position and appearance of the nuclei within a radius of .7 to .8 mm. of the injury. Miehe finds the maximum distance to which such reactions may extend is often as much as 1.8 mm. Figures 1 and 8 by Nestler show, in every cell in the vicinity of the injury, the nuclei lying in a dense mass of cytoplasm against or in close proximity to the cell wall nearest the injury. These figures suggest a general

flow of the protoplasm in the direction of the injury. Not only does the position of the nuclei change, but their appearance also, as is shown by Miehe's figures 8a and 8b, and figure 51 of my preparations. Miehe describes the nuclei of the cells lying near the injury as being very coarse and granular in appearance. In his figures 5, 6 and 7, each nucleus appears much denser and more granular on the side toward the injury, as if a stream had passed through the nucleus in the direction of the injury, carrying the granules and particles in the nucleus with it.

It is to be noted, however, that the observations of the above investigators, with the exception of Miehe, were made several hours and even days after the injuries were made, and they do not record any immediate changes such as are shown in figure 51 of my preparations. Miehe, on the other hand, describes marked changes in the cells and nuclei thirty minutes after injury. In the light of these observations, it seems questionable whether any living tissue, such as the root tips of *Allium* and *Vicia* used by Lundegårdh (37) and Schaede (68), which has first to be sectioned, is suitable for study of nuclear and cell division in living material. It is questionable whether any living root tip can be sectioned thin enough for study under the high powers of the microscope by the methods commonly in practice and still not show injury effects in the nuclei and cytoplasm. Although Lundegårdh and Schaede undoubtedly selected cells whose nuclei and cytoplasm showed no signs of injury, it is not improbable, in the light of Miehe's and Nestler's observations, that microscopically invisible changes had been initiated as a result of the sectioning, which later affected the appearance of the nucleus and cytoplasm.

The photographs shown in PLATE 13, as noted before, are representative of the changes in the nuclei in the antheridial filament cells of *Nitella* and *Chara* as they divide. If we compare the very earliest prophase changes with the changes that occur in the appearance of the nuclei as a result of being mounted in water and sugar solutions, as described for PLATE 12, we are impressed with a certain, and perhaps misleading similarity of the two processes. In the different series shown in PLATE 12, the dense elements in the resting nuclei apparently become less dispersed and more aggregated until large granules, bodies and strands are visible; and similarly in the earliest prophases there

is perhaps a decrease in the dispersion of the dense elements, accompanied by a tendency to aggregation and increased size of the dispersed elements. However, much further study is needed to show the real nature of these changes and their relation to the later stages of chromosome formation.

The presence of an irregular, coarsely granular reticulum with more or less uniform nucleoles in the resting nuclei of aceto-carmine and unfixed and unstained preparations of *Nitella* and *Chara* is strong evidence against the origin of the spireme and chromosomes from an aggregated nucleole-like mass of material. While the highly stainable, aggregated body which is so often present in resting nuclei of fixed and stained preparations frequently decreases in size and appears to lose some of its contents in the prophases, I find no adequate evidence that its material goes to make the spireme and chromosomes. In the late prophases, as shown in figures 10 and 12, the aggregated mass has apparently disappeared, and a more or less rounded, single nucleole is present. In figure 9, on the other hand, a deeply stained aggregated mass of material is still present, although the spireme segments are fully formed. It is difficult to differentiate a true nucleole in the aggregated mass seen in fixed preparations, although definite rounded bodies are shown in figures 2, 3, 29, and 31. The staining reaction of the mass as a whole is generally the same throughout, but a number of preparations have been found (FIGS. 29, 30, 31) in which a large body has stained intensely red in contrast to the violet color of the surrounding fragments. In the early prophase shown in figures 34 and 35, the staining reaction of the granules and fragments is quite different from that of the round, nucleole-like bodies. In Flemming's triple stain, the granules, fragments and strands are a deep violet in contrast to the ruby red of the rounded nucleole-like bodies.

In the telophases the chromosomes are transformed into typical dispireme threads, whose staining reaction is distinct from that of the rounded bodies which begin to appear in the daughter nuclei at this stage. While the evidence from fixed and stained material is not at all complete, the conditions found in unfixed and unstained and aceto-carmine preparations of resting and prophase nuclei point to the origin of the spireme and chromosomes in *Nitella* and *Chara* from an irregular and somewhat granular reticulum.

The view of Zimmermann, Kaiser, Debski, and Riker, that the numerous deeply stained irregular and rounded bodies in the cytoplasm of *Nitella* and *Chara* are extra-nuclear material or bodies extruded from the nucleus, and particularly formed by the disintegration of the nucleole during division, seems improbable in the light of the conditions shown in figures 19, 23, 25, 27, and 28. It is difficult to explain the large masses of bodies or granules here shown as coming from the nucleus, and particularly the nucleole, during mitosis, unless, of course, they are assumed to grow and divide when they get into the cytoplasm. The volume of the granules lying in the cytoplasm of figures 19 and 23 appears to be almost equal to that of the chromatin of the entire nucleus, and the mass of highly stainable material present at the poles of the spindle shown in figure 28 is equal in volume to the whole group of chromosomes in the equatorial region. According to the view that these bodies are extra-nuclear nucleoles extruded from the nucleus during mitosis, we would naturally expect to find them more numerous during division stages than in resting cells, unless we assume that they persist from one cell generation to another. Yet in figures 23, 24, and 25 of resting cells, fully fifty deeply stained bodies of various sizes and shapes are present, while in the dividing cells shown in figures 11, 12, 13, 16a, 17, 20 and 21, comparatively few such bodies are visible. The contrast between the number of bodies in resting and dividing cells is not always as marked and general as noted in the cells just referred to, but such cases indicate that highly stained chromatic bodies may occur in the cytoplasm regardless of whether the nucleus is undergoing division or not. The appearance of the bodies in the equatorial region of the spindle shown in figure 27 is somewhat like that of nucleoli, but their large size and number again render it highly improbable that they are of nucleolar origin. The fact that these granules are not destroyed by fixatives containing acetic acid, coupled with their difference in size and staining reaction from chondriosomes as commonly shown, indicates that they are of a different nature from these latter bodies.

While it is true that small bodies or granules frequently occur near or in contact with the nuclear membrane during prophase and at the poles of the spindles in metaphase and anaphase,

they are not, it seems to me, to be regarded as centrosomes, as held by Schottländer and Kaiser. As previously noted in figure 1, a number of granules lie near the nuclear membrane, and two of them appear somewhat like two large centrosomes that have resulted from a previous division. In figure 11, two small and two relatively large bodies lie at the opposite sides of the nucleus, while the two bodies lying at the poles of the spindle shown in figure 17 are very irregular in shape and as large in size as some of the small chromosomes in the equatorial region. A comparison of the granules and bodies in these figures with those shown in figures 16a, 16b, 18, and 19, shows little difference in size, shape and staining capacity between the two. It seems evident from a comparison of the preparations that the bodies frequently occurring near or in contact with the nuclear membrane and at the poles of the spindle are of the same type as the bodies lying variously scattered in the cytoplasm, and differ from the latter only in size and position in the cell. No astral rays have been seen in connection with these bodies, and the spindle appears to be formed independently of their presence. Some evidence of radial kinoplasm may be seen in figure 11, and faint indications of polar caps are present in the cell of an antheridial filament shown in figure 40; but I have not been able to determine with certainty the mode of origin of the spindle in *Nitella* and *Chara*.

The difficulty of using photographic methods on cytological material, as noted before, particularly living cells and aceto-carmine preparations, is evident in the figures shown in PLATES 12 and 13. The figures in PLATE 13 are inadequate for bringing out the cytological details of mitosis, but they show certain of the mass changes that occur in the colloidal protoplasm during division. It is becoming increasingly evident from the study of matter in the colloidal state that the so-called cell organs, nucleole, chromatin granules, linin, nuclear sap, etc., are elements in a complex colloidal system, and possibly in mitosis pass from one state to another. In the light of this evidence and the fact that our attention is becoming more and more centered on the mass changes in the cellular colloid system as it grows and divides, it is certainly worth while to give renewed attention to nuclear and cell division in living cells, and material as little modified by fixation as possible. The antheridial filaments of the Characeae, in my opinion, are specially

favorable material for such study. I am continuing my studies on this material and regard my results so far as preliminary. The possibility of checking the appearances obtained in fixed and stained material by the study of living cells and *intra-vitam* staining, it seems to me, is of great importance.

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Explanation of plates 10–13

All figures were drawn with the aid of a Spencer camera lucida and a Zeiss 2 mm. aprochromatic objective N. A. 1.30 and compensating oculars 6, 8, 12 and 18. The approximate magnifications are given with each figure. The photographs in plates 12 and 13 were made with the same objective and compensating oculars 6, 8 and 12, with a Leitz Liliput arc light as the source of illumination. The approximate magnification is given with each photograph.

Plate 10

FIG. 1. Nodal cell of *Nitella gracilis* showing a mass of densely stained material at the center of the nucleus and numerous irregular granules in the cytoplasm. × 1800.

FIGS. 2 and 3. Nuclei of mature eggs of *N. gracilis* showing similar masses of deeply stained material in the center. Vacuolated nucleoli lie close to the dense masses in the center. × 2400.

FIG. 4. Nucleus of an apical cell of *N. gracilis* with four deeply stained, rounded bodies in the nuclear reticulum. × 1800.

Fig. 5. Nucleus of an egg of *N. gracilis* showing chromatic reticulum as in FIG. 4, and knob-like projections of the aggregated mass at the center. × 1250.

FIG. 6. Nucleus of a nodal cell of *N. gracilis* showing chromatic reticulum as in FIGS. 4 and 5, and seven densely stained bodies closely grouped together. × 1800.

FIG. 7. Nucleus of an apical cell of *N. gracilis* with numerous dark bodies in the nuclear reticulum and knob-like projections from the central mass of material. × 1800.

FIG. 8. Nucleus of an apical cell of *N. gracilis* drawn from an aceto-carmine preparation with a rounded nucleole and a granular reticulum. × 1800.

FIG. 9. Late prophase nucleus of a young manubrium of *Chara sp.* showing irregular spireme segments together with a deeply stained, irregular mass of material. × 2400.

FIG. 10. Late prophase nucleus of an apical cell of *Chara sp.* showing nodular spireme and a somewhat constricted nucleole lying next to the nuclear membrane. × 3400.

FIG. 11. Late prophase stage in a cell of the 2-cell stage of an antheridium of *N. gracilis*. The nucleus is filled with an even, convoluted spireme, and the cytoplasm around the nucleus suggests a radial arrangement. Two large and two small granules lie at the opposite poles of the nucleus. × 1800.

FIG. 12. Late prophase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing a round nucleole and approximately 32 spireme segments. × 3400.

FIG. 13. Polar view of the first division of the nucleus in the 1-cell stage of an antheridium of *N. gracilis* showing 34 chromosomes and 7 chromatic bodies. × 1250.

FIG. 14. Polar view of an equatorial plate of a nodal cell of *N. gracilis* showing 34 chromosomes. × 1800.

FIG. 15. Polar view of late prophase or equatorial plate in an antheridial filament cell of *N. gracilis*. × 1800.

FIGS. 16a and 16b. Early metaphase in a cell of the 8-cell stage of an antheridium of *N. gracilis*. × 3400.

FIG. 17. Early anaphase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing numerous granules of various sizes in the cytoplasm and the spindle converging at the poles toward two large, deeply stained bodies. × 2400.

FIG. 18. Late anaphase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing the broad, barrel-shaped spindle. × 2400.

FIG. 19. Dispireme in a cell of the 4-cell stage of an antheridium of *N. gracilis*. The daughter nucleus at the upper end of the cell is almost obscured by large, deeply stained bodies in the cytoplasm. Numerous similar bodies are also present near the equator of the spindle. × 2400.

Plate 11

FIG. 20. Late telophase in a nodal cell of *N. gracilis* showing the thickening of the spindle fibers in the equatorial region. In the center of the daughter nuclei are large masses of deeply stained material. × 2400.

FIG. 21. Early cell plate stage in a cell of the 4-cell stage of an antheridium of *N. gracilis*. The cell plate has not yet reached the walls of the cell. × 1800.

FIG. 22. Late cell plate stage in a nodal cell of *N. gracilis*. × 1800.

FIG. 23. One-cell stage of the antheridium of *N. gracilis* showing approximately fifty deeply stained granules and bodies of various shapes and sizes lying in a somewhat semi-circular group around the nucleus in the upper part of the cell, marked *x*. × 1250.

FIG. 24. Tip cell of a young oogonium of *N. gracilis* after the first 'Wendungszelle' has been cut off, showing a mass of granules and bodies on the side of the nucleus towards the external surface of the cell. Practically no granules and bodies are present in the 'Wendungszelle'. × 1250.

FIG. 25. Egg cell of *N. gracilis* with two 'Wendungszellen' showing approximately fifty deeply stained and variously shaped granules or bodies in the upper part of the egg cell. Very few bodies are present in the 'Wendungszellen.' × 1800.

FIG. 26. Nodal cell of *N. gracilis* fixed in Benda's fluid and stained according to Benda's method for chondriosomes. The large bodies or granules are distinct from the chondriosomes. × 1800.

FIG. 27. Late anaphase stage in a nodal cell of *Chara sp.* showing eleven large dense bodies in the equator of the spindle. × 2400.

FIG. 28. Equatorial plate stage in an apical cell of *Chara sp.* showing dense masses of bodies of various sizes and shapes at the poles of the spindle. The bodies are more numerous at the upper pole of the spindle. × 3400.

FIGS. 29–33. Nuclei from resting cells of the antheridial filaments of *N. gracilis* showing numerous deeply stained bodies in the nuclear reticulum. × 2400.

FIGS. 34–36. Prophase changes in the nuclei of antheridial filaments of *N. gracilis*. × 2400.

FIG. 37. Nucleus of an antheridial filament cell of *Chara sp.* with a nodular spireme. × 2400.

FIG. 38. Late prophase nucleus of *Chara sp.* showing numerous spireme segments, three of which appear longitudinally split. × 3400.

FIGS. 39 and 40. Late prophase nuclei in antheridial filament cells of *N. gracilis* showing some evidence of polar caps. × 2400.

FIG. 41. Equatorial plate stage in an antheridial filament cell of *N. gracilis*. The chromosomes lie diagonally placed in the cell. × 2400.

FIGS. 42 and 43. Polar views of equatorial plate stage in aceto-carmine preparations of antheridial filament cells of *Chara sp.* showing variation in size and shape of the chromosomes in different cells. × 2800.

FIG. 44. Equatorial plate stage in the terminal cell of an antheridial filament of *Chara* sp. showing the equatorial plate parallel to the long axis of the filament. × 2400.

FIG. 45. Early anaphase in a cell of an antheridial filament mounted in aceto-carmine. No spindle fibers are visible. × 2800.

FIG. 46. Metaphase or early anaphase in an antheridial filament cell of *N. gracilis*. × 2800.

FIG. 47. Late anaphase in an antheridial filament cell of *N. gracilis* with the division figure diagonally placed in the cell. × 2400.

FIG. 48. Later anaphase in two antheridial filament cells of *Chara sp.* showing barrel-shaped spindle lying in a diagonal position in the cell. × 2400.

FIG. 49. Early cell plate in two antheridial filament cells of *Chara sp.* The telophase nuclei appear flattened against the end walls of the cells. × 2400.

FIG. 50. Late cell plate stage in an antheridial filament cell of *N. gracilis*. The cell plate is completely formed across the equator of the cell. Spindle fibers are still present, however. × 2400.

Plate 12

FIG. 51. Antheridial filament of *Chara sp.* showing change of shape, position and appearance of the nuclei as a result of mechanical injury. × 1000.

FIGS. 52–57. Portion of an antheridial filament of *Chara sp.* mounted in tap water. × 1000. FIG. 52. Ten minutes after mounting. FIG. 53. Fifteen minutes after mounting. FIG. 54. Twenty minutes after mounting. FIG. 55. Twenty-five minutes after mounting. FIG. 56. Thirty minutes after mounting. FIG. 57. Thirty-five minutes after mounting.

FIGS. 58–61. Portion of an antheridial filament of *Chara sp.* mounted in tap water. × 1000. FIG. 58. Fifteen minutes after mounting. FIG. 59. Twenty minutes after mounting. FIG. 60. Thirty minutes after mounting. FIG. 61. Thirty-five minutes after mounting.

FIGS. 62–67. Portion of an antheridial filament of *Chara sp.* mounted in once -distilled water. × 1000. FIG. 62. Five minutes after mounting. FIG. 63. Ten minutes after mounting. FIG. 64. Fifteen minutes after mounting. FIG. 65. Twenty-five minutes after mounting. FIG. 66. Thirty minutes after mounting. FIG. 67. Forty minutes after mounting.

FIGS. 68–71. Portion of an antheridial filament of *Chara sp.* mounted in one and one-half per cent cane sugar solution. × 1000. FIG. 68. Five minutes after

mounting. FIG. 69. Thirty minutes after mounting. FIG. 70. Sixty minutes after mounting. FIG. 71. Two hours after mounting.

FIGS. 72–76. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution. × 1000. FIG. 72. Five minutes after mounting. FIG. 73. Fifteen minutes after mounting. FIG. 74. Thirty minutes after mounting. FIG. 75. One hour after mounting. FIG. 76. Two hours after mounting.

FIGS. 77–80. Portion of an antheridial filament of *Chara sp.* mounted in five per cent cane sugar solution. × 1000. FIG. 77. Five minutes after mounting. FIG. 78. Twenty minutes after mounting. FIG. 79. One hour after mounting. FIG. 80. Six hours after mounting.

FIG. 81. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution and photographed five minutes later. × 1000.

FIG. 82. Same filament transferred to aceto-carmine and photographed fifteen minutes later. × 1000.

FIG. 83. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution and photographed ten minutes later. × 1000.

FIG. 84. Same filament transferred to aceto-carmine and photographed ten minutes later. × 1000.

FIGS. 85–90. Portion of an antheridial filament of *Chara sp.* mounted in aceto-carmine. × 1000. FIG. 85. Five minutes after mounting. FIG. 86. Thirty minutes after mounting. FIG. 87. Three hours after mounting. FIG. 88. Six hours after mounting. FIG. 89. Twelve hours after mounting. FIG. 90. Thirty hours after mounting.

Plate 13

FIG. 91. Terminal end of an antheridial filament of *Chara sp.* mounted in aceto-carmine and photographed one hour after mounting. The appearance of the nuclei suggests the resting stage. The nuclei are more or less uniformly dark, but some differentiated light and dark elements are visible. × 1800.

FIG. 92. Four terminal cells of an antheridial filament of *Chara sp.* photographed one-half hour after mounting in aceto-carmine. The nuclei are less uniformly dark than in the previous figure, and show well differentiated dark elements that appear like strands, irregular bodies, and granules. Possibly an early prophase. × 1800.

FIG. 93. Four terminal cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine. The appearance of the dark elements suggests a coarse reticulum lying in an optically hyaline ground substance. Perhaps a later stage than FIG. 92. × 1950.

FIG. 94. Terminal cell of an antheridial filament of *Chara sp.* photographed thirty minutes after mounting in aceto-carmine in whose nucleus appears the suggestion of an irregular reticulum lying in an optically homogeneous continuum. × 1000.

FIG. 95. Two terminal cells of an antheridial filament of *Chara sp.* photographed forty-five minutes after mounting in aceto-carmine. The appearance of the nuclei suggests possibly a later prophase stage than the previous figures. × 1950.

FIG. 96. Two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. In the terminal nucleus may be counted approximately eleven large, dense elements that appear more or less connected. Obviously a later prophase stage than shown in any of the preceding figures. × 1950.

FIG. 97. Terminal cell of an antheridial filament of *Nitella sp.* photographed one and a half hour after mounting in aceto-carmine. There are approximately nine dark, dense and conspicuous elements in the nucleus which appear somewhat twisted and curved, suggesting perhaps chromosomes. Nuclear boundary evidently still present. × 1950.

FIG. 98. Terminal cell of an antheridium filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine. Approximately seven more or less connected dense elements are visible. × 1800.

FIG. 99. Terminal cell of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. Perhaps a later prophase stage than shown in the previous figure. × 1250.

FIG. 100. Three cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine showing rounded masses of band-like dense elements. × 1250.

FIG. 101. Three terminal cells of an antheridial filament of *Nitella sp.* showing three thick, dense bands extending diagonally across the central cell. × 1950.

FIG. 102. Terminal cell of an antheridial filament of *Nitella sp.* photographed two hours after mounting in aceto-carmine. In the nucleus may be seen a number of dense chromosome-like elements that appear more or less connected. × 1950.

FIG. 103. Terminal cell of an antheridial filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine whose nucleus appears very similar to that shown in the previous figure. × 1950.

FIG. 104. Terminal cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine showing the chromosomes as round bodies. Possibly polar views. × 1950.

FIG. 105. Portions of two antheridial filaments of *Nitella* sp. photographed one hour after mounting in aceto-carmine. Equatorial plate shown in cell *A*. The chromosomes marked *2* and *3* in cell *A* show a light central line throughout their length. A similar appearance is visible in the chromosomes marked *1* in cell *B*. × 2400.

FIG. 106. Two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. Nuclei in early anaphase stage. Chromosome groups almost parallel to the long axis of the filament. × 2200.

FIG. 107. Late anaphase stage in cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. × 1800.

FIG. 108. Two terminal cells of an antheridial filament of *Chara sp.* photographed twenty minutes after mounting in tap water. Fiber-like and cell plate elements are visible between the daughter nuclei. × 1000.

FIG. 109. Late anaphase stage in the terminal cell of an antheridial filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine. × 2200.

FIG. 110. Possibly so-called diaster stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine. × 1800.

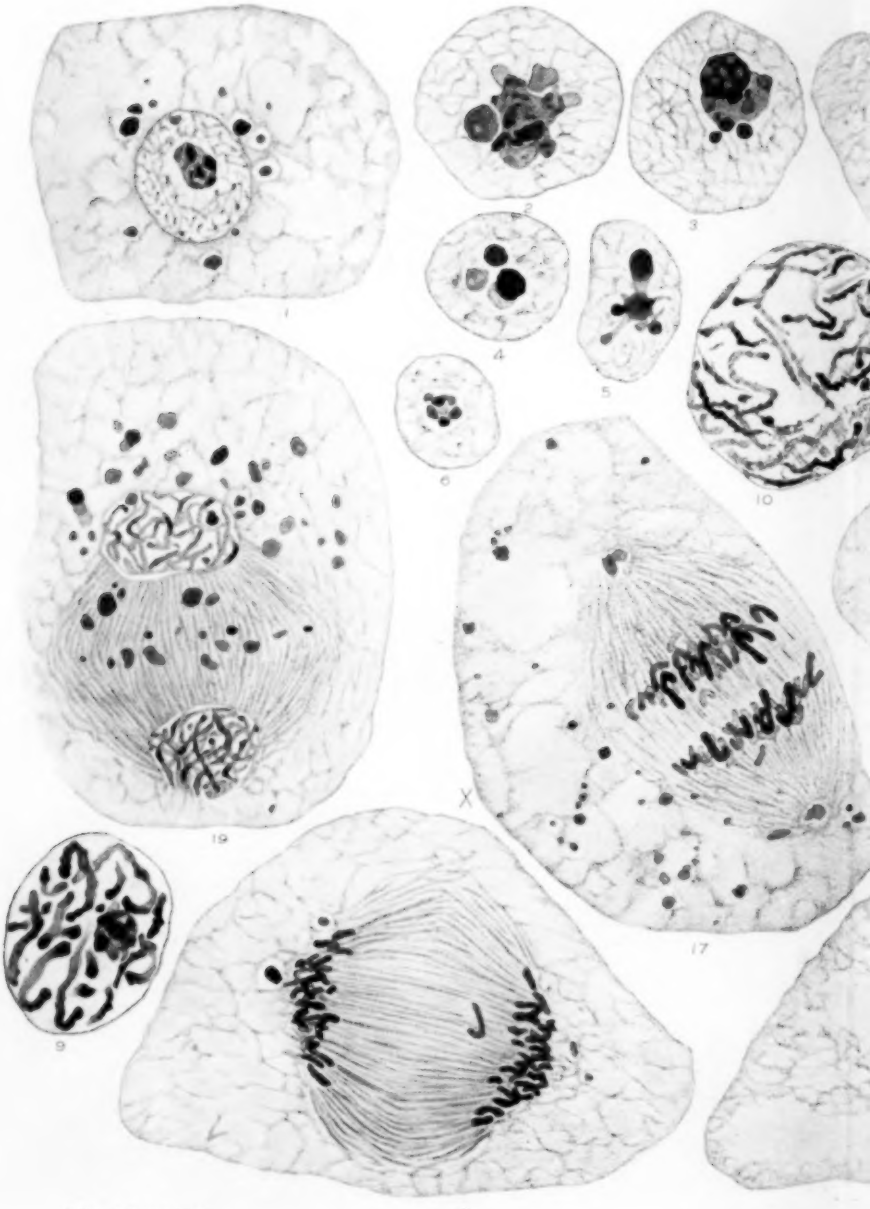
FIG. 111. Late anaphase stages in cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine in which the daughter groups of chromosomes lie in diagonally opposite corners of the cells. × 1800.

FIG. 112. Early telophase stages in two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. The appearance of the nuclei suggests the so-called dispireme stage of fixed and stained preparations. × 1800.

FIG. 113. Later telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting. × 1800.

FIG. 114. Late telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in which cell plate elements are visible between the daughter nuclei. × 1800.

FIG. 115. Late telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting, in which the nuclei have somewhat the appearance of so-called resting nuclei. The cell walls are completely formed between the daughter nuclei. × 1650.



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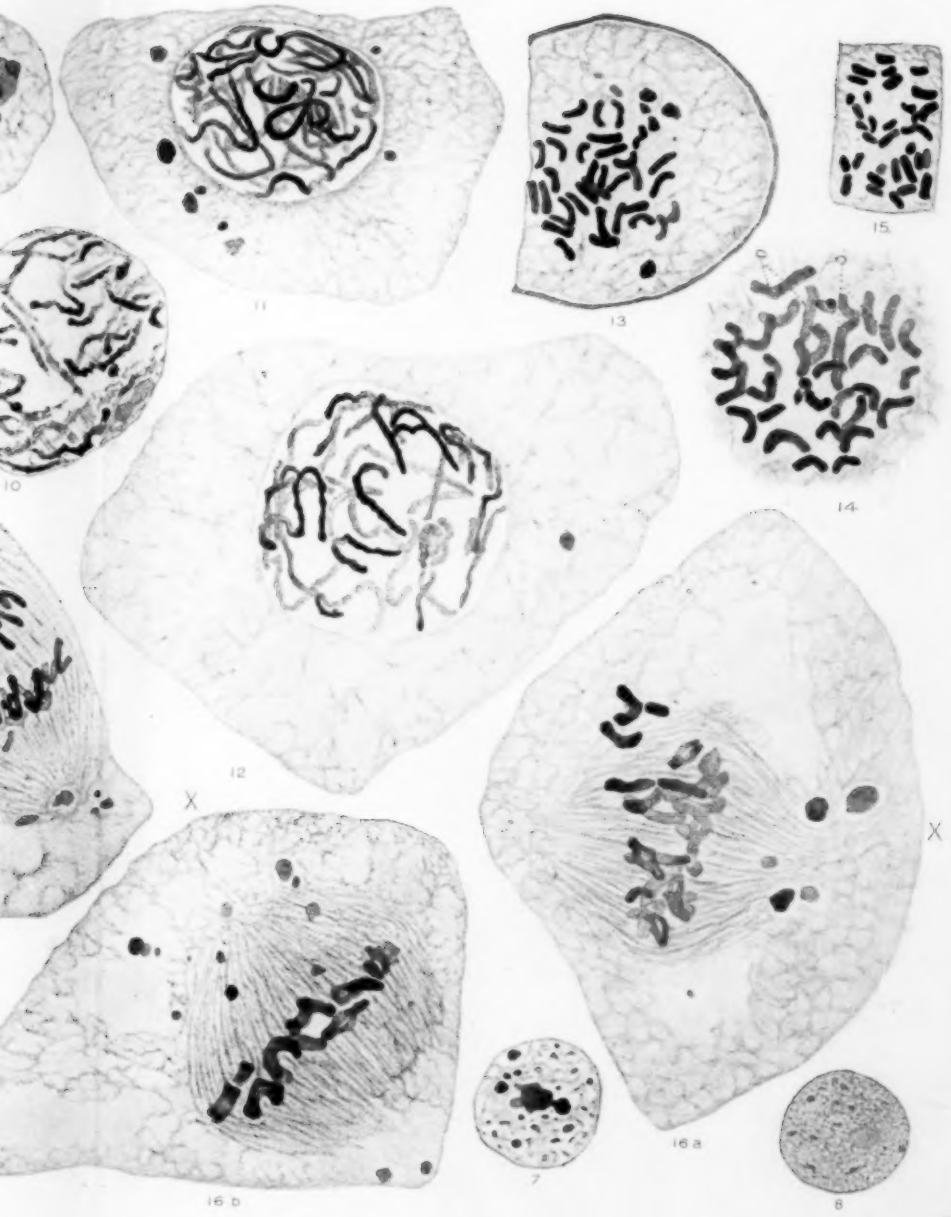
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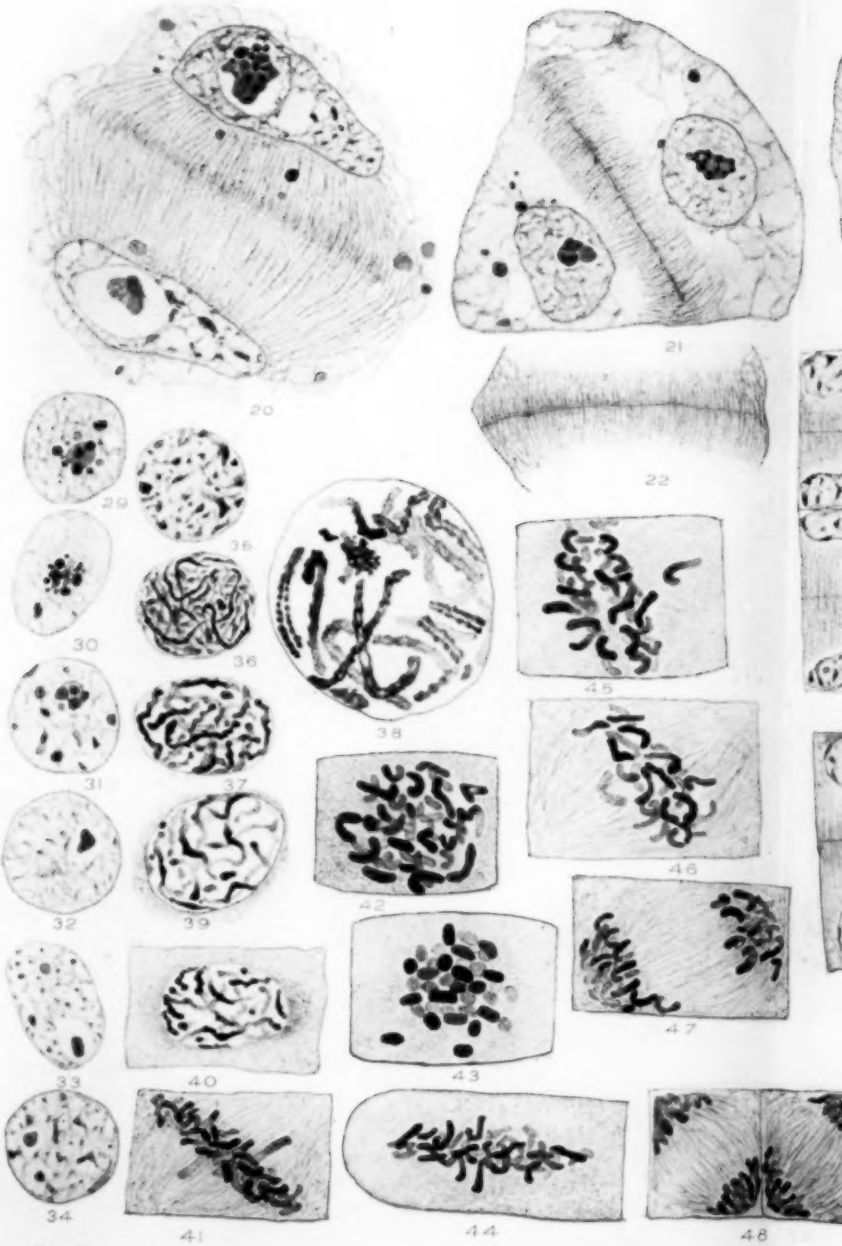

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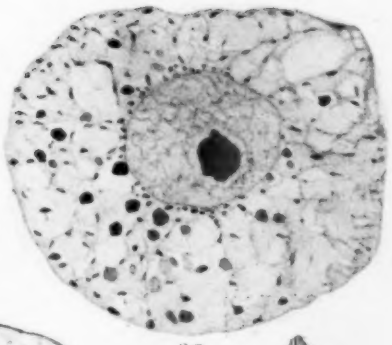

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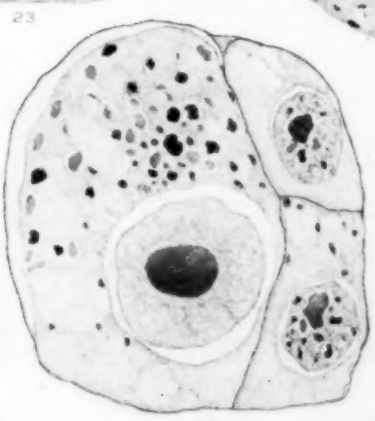

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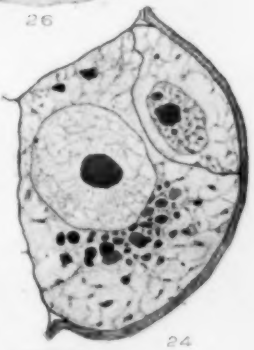

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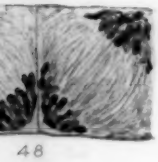

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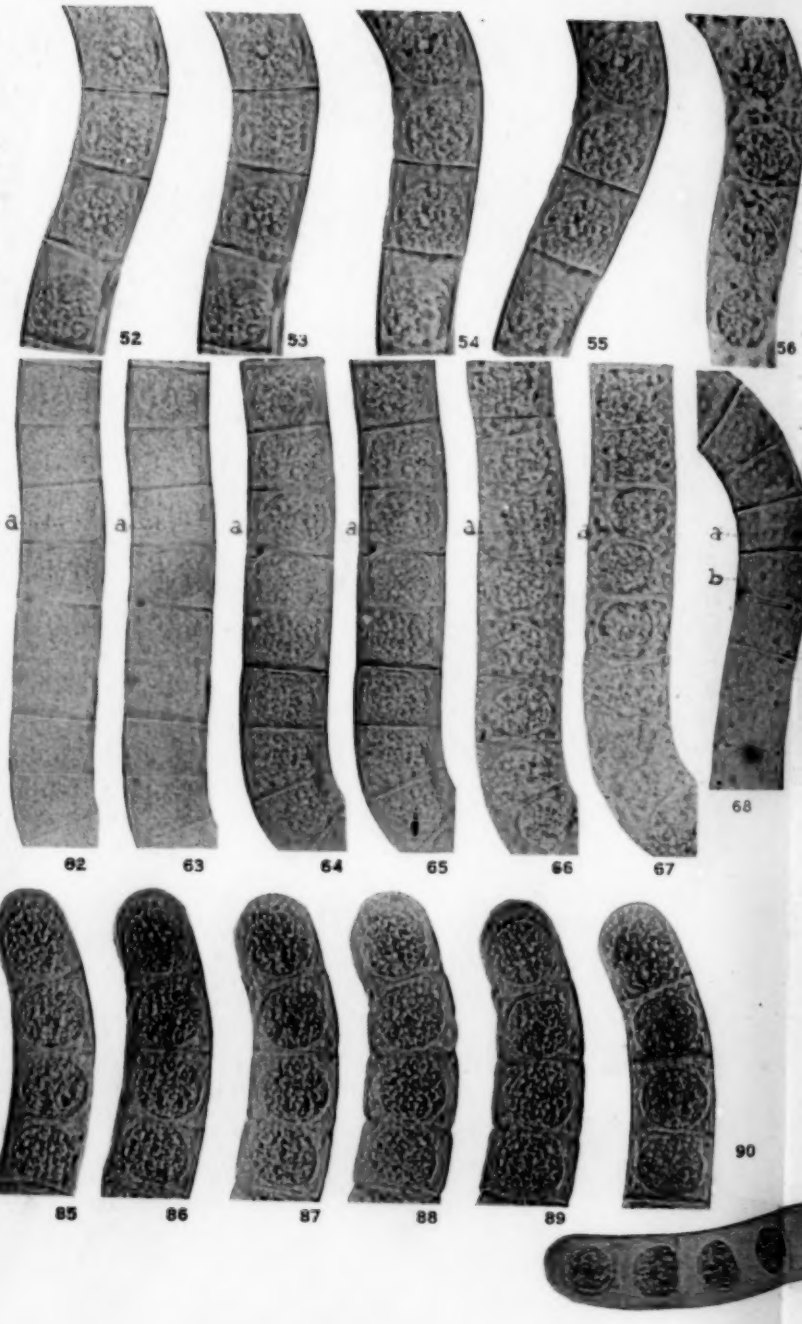

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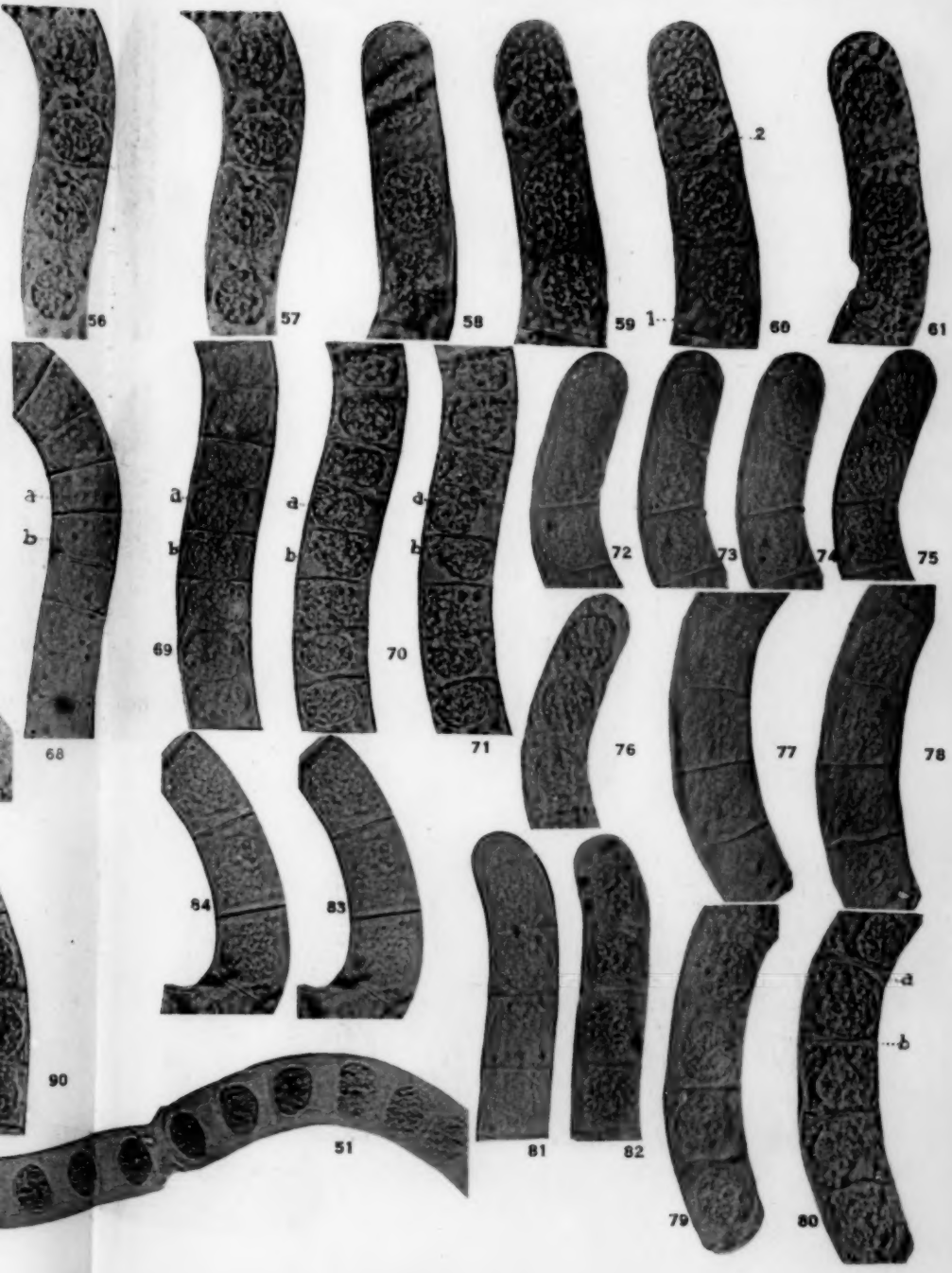
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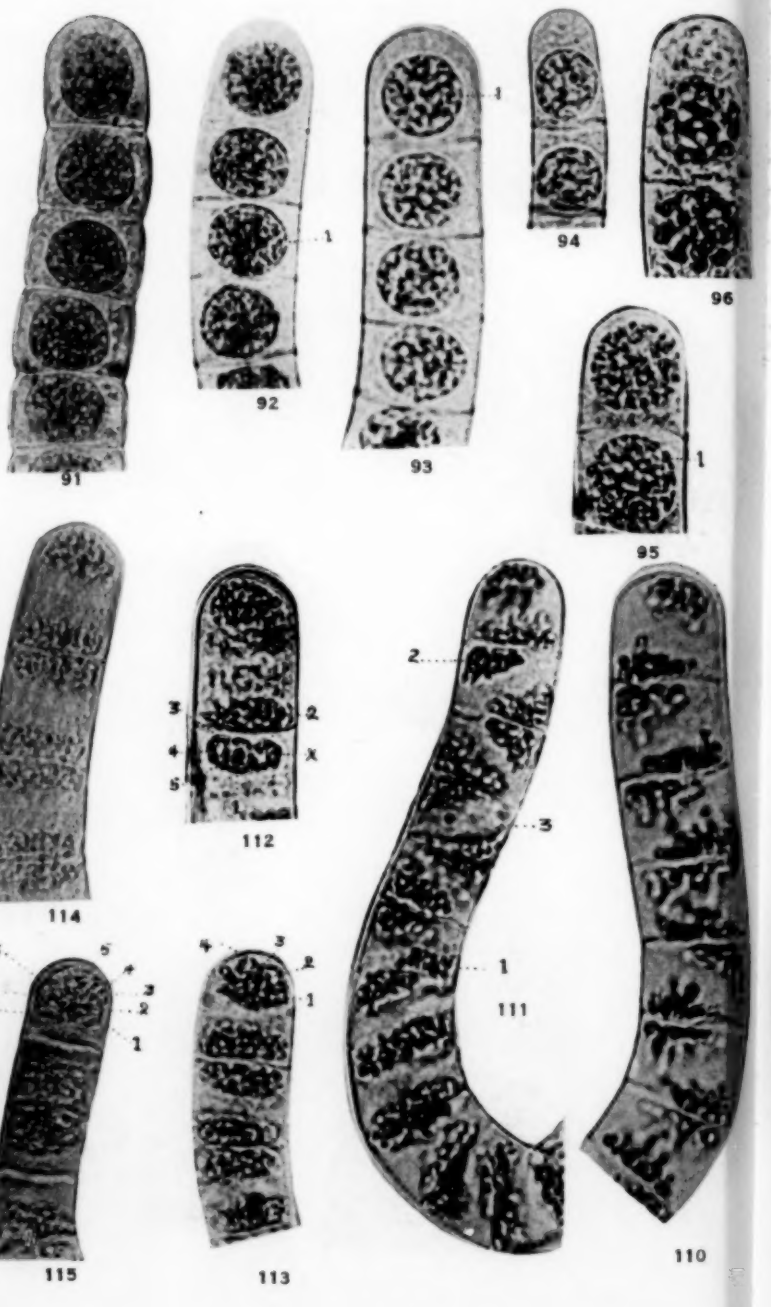
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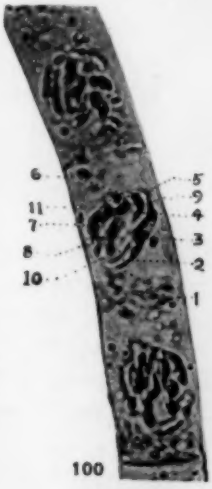

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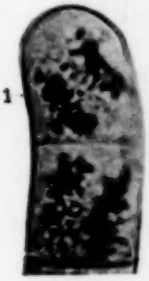

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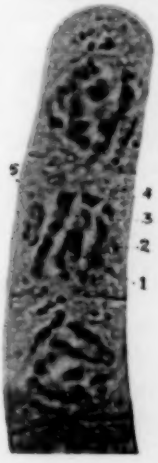

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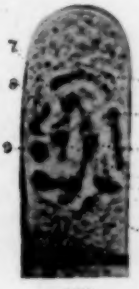

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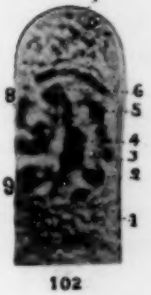

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UNIV. OF MICH.

Persistence of the antipodal tissue in the development of the seed of maize[1]

PAUL WEATHERWAX

(WITH SIX TEXT FIGURES)

It has long been known that the three antipodal cells present at the time of the organization of the embryo sac of maize (*Zea Mays* L.) undergo a series of divisions before the time of fecundation, resulting in the formation of a compact antipodal tissue made up of many cells. The limited number of investigations that have been made indicate that this is a characteristic common to many, if not all, grasses.

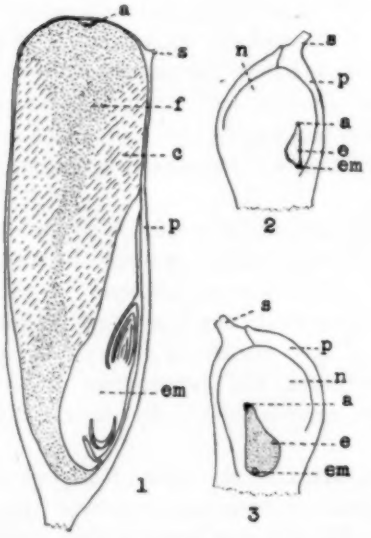
Although the function of this antipodal tissue is probably of negligible importance, it is of morphologic interest because it constitutes a gametophytic development unusual in the Angiosperms. The fate of the antipodal cells has usually been disregarded, or disposed of in a few words, in embryological studies of the cereals in recent years, the assumption or the observed fact being that they are early absorbed by the rapidly developing endosperm.

Golinski (1), reviewing the literature of the time of his publication (1893), and adding his own observations, reported that the antipodals were absorbed. He did not have a first-hand knowledge of the embryology of maize, but criticized the work of Westermaier, who reported certain differences between the antipodals of maize and those of such grasses as wheat and barley. Jensen (3) found that the antipodals in wheat were absorbed. Miller (5) reported the same for maize, but two of his figures (*A* and *B*, *pl. 32*) might be otherwise interpreted. Accepting the published views of others, and judging from superficial appearances, the writer (8) has also stated that the antipodals are absorbed in maize.

A recent examination, however, of some sections of nearly mature grains of ordinary field corn showed, in the region of the "dent," some small groups of cells distinctly different from those of the nucellus or of the ordinary endosperm (FIGS. 1, 4). This led to a more thorough examination of an old series of

[1] Publication no. 24 of the Waterman Institute for Research.

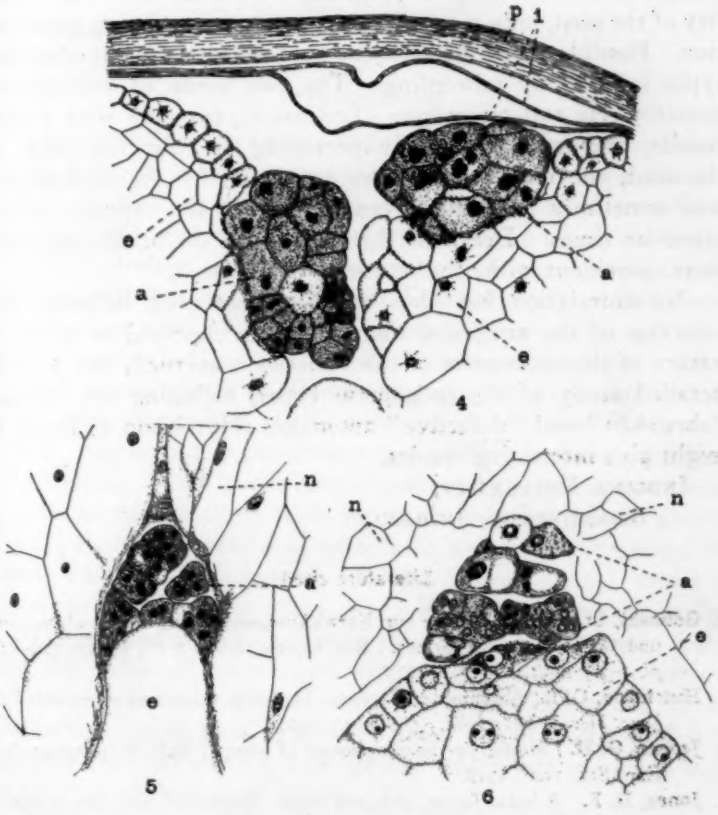
embryological slides, and of new ones prepared for this purpose, to determine the nature of the tissue in question. It was found that, without doubt, the antipodal tissue often persists to the maturity of the grain. FIGURES 1–6 show the position and appearance of this tissue at different times in the development of the grain. The mature embryo sac has been figured several times (Weatherwax 7, *fig. 1, pl. 23*; 8, *fig. 13, pl. 7*; Miller 5, *pl. 23*).



FIGS. 1–3. Longitudinal sections of caryopses of different ages. FIG. 1. Almost mature. FIG. 2. Free-nucleate stage of endosperm development; embryo of only three or four cells. FIG. 3. Intermediate stage of development. *a*, antipodal tissue; *p*, pericarp (ovary wall); *n*, nucellus; *e*, endosperm; *f*, floury endosperm; *c*, corneous endosperm; *em*, embryo; *s*, base of style.

At the time of the maturity of the embryo sac the antipodal cells usually number 25 or more, these having arisen by the division of the original three. In at least the earlier of these divisions the nuclei divide mitotically, but in later stages the division is amitotic, and cells with two, three, or more nuclei are common. After fecundation the antipodal tissue is sometimes soon absorbed by the developing endosperm, but in too many instances for it to be regarded as constituting merely an anomaly, its cells continue to divide and grow slowly at the expense of the impoverished tissue of the nucellus. Occasionally also it may prey upon the nearby cells of the endosperm (FIG. 6). Some of its cells are filled with a dense, deeply-staining protoplasm; others have large vacuoles. The cells are loosely joined together, the one or more groups resembling the loosely organized colonies of some thallophytes. As many as five or six nuclei have been observed in some cells, and fragmentation of the nuclei continues until the seed is practically mature.

Morphologists have pictured the grain of corn as consisting of three distinct generations of tissue: the maternal sporophyte, the embryonic filial sporophyte, and the problematical endo-



FIGS. 4–6. The antipodal masses indicated in FIGS. 1–3, and numbered in the same order. *p*, pericarp; *i*, integument; *a*, antipodal tissue; *e*, endosperm (xeniophyte); *n*, nucellus; The chalazal end of the young endosperm (*e* in FIG. 5) has a very large vacuole. The apparent disorganization in a few cells at the upper end of the endosperm in FIG. 6 probably indicates at least a temporary invasion of the endosperm by the antipodal tissue.

sperm. To these must now be added the occurrence sometimes of a small portion of the true gametophytic endosperm, the exact homologue of the normal endosperm in most Gymnosperms.

Following fecundation the inside of the grain is the scene of a

life-and-death struggle among these four generations of tissue. The aged and attenuate maternal sporophyte tissue of the nucellus always succumbs, and the young filial sporophyte always wins in the long run, pausing for a while after the maturity of the seed, only to complete its devastation during germination. Possible exceptions may be noted in some of the aberrant types isolated by inbreeding. The two kinds of endosperm, gametophyte and xeniophyte (Trelease 6), compete with varied results, the latter ordinarily persisting to the maturity of the seed, the former sometimes consumed by the xeniophyte, and sometimes persisting, even slightly at the expense of the latter at times. Death of the endosperm doubtless precedes, or is coincident with, the maturity of the seed.

No correlation has thus far been observed between the behavior of the antipodal tissue and the chemical or physical nature of the endosperm of the varieties concerned, but a more detailed study of the endosperm types, including the various "shrunken" and "defective" anomalies (Hutchison 2; Jones 4) might give interesting results.

INDIANA UNIVERSITY,
BLOOMINGTON, INDIANA.

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The production of intumescences upon apple twigs by ethylene gas[1]

RAYMOND H. WALLACE

(WITH PLATES 14 AND 15)

INTRODUCTION

During the past two years I have made numerous experiments on the effect of ethylene gas upon apple twigs and have obtained results which it seems advisable to publish in preliminary form, leaving until later the complete report on the whole subject.

That ethylene induces characteristic reactions in living organisms is well known. Knight and Crocker (1913) showed very clearly that most of the toxic effects of illuminating gas upon plants are probably due to small amounts of ethylene. All of the injurious effects of illuminating gas on vegetation which have been reported in the literature since the time of Girardin (1864) may be due in the greater part to the physiological effects of ethylene. Some of the effects induced by ethylene have been given commercial application during the past few years. Denny (1924) found the yellowing of lemons was greatly hastened by small amounts of ethylene. R. B. Harvey (1925) found it excellent for blanching celery, while Rosa (1925) reports having used it successfully for removing chlorophyll from tomato fruits and for inducing more rapid sprouting in dormant potatoes. Another important application of ethylene is its use as an anaesthetic in surgical practice. From the data reported it is obvious that ethylene is a substance which can induce a wide variety of physiological effects.

The earliest studies on the effect of illuminating gas upon vegetation dealt almost entirely with the killing of trees, shrubs, and herbs, by either accidental or experimental gas leaks. Kny (1871) killed trees by exposing their roots for several weeks to a continuous flow of illuminating gas. Defoliation on the part of the lindens was the first evidence of injury. Späth and Meyer (1873) performed more extensive experiments with a greater number of species and likewise found that defoliation and death results from exposing the roots of trees to gas.

[1] Contributions from the Department of Botany of Columbia University no. 345.

Wehmer (1900) gives perhaps the most striking case on record of gas poisoning of trees. Thirteen elms located on either side of a leaking gas main showed a correlation between intensity of injury and distance from the leak. The effect became evident in late winter when large pieces of bark, in some cases several feet in length, dropped off. Twigs and buds remained normal even on trees whose trunks were completely girdled. During the spring some of the trees developed a complete set of foliage leaves while others did not. He attributed the wide zone of damage to the hard crust on the ground which caused the gas to spread farther beneath the surface than it otherwise would.

The relative susceptibility of various plants to gas poisoning has received no little attention from investigators. Lackner (1873) divided plants into those that are injured and those that are not when grown in a room in which illuminating gas is burned. Neljubow (1901) was able to show that impurities in the air were responsible for the horizontal growth or diageotropism of etiolated pea seedlings so often exhibited by plants grown in the laboratory. He found that either ethylene or acetylene would cause it. He established that diageotropism, increased growth in thickness, and decreased growth in length constituted a triple response to impurities in laboratory air. One part of ethylene to 1,000,000 parts of air gave the response, while one part to 4,000 of air caused the death of the seedlings.

Crocker and Knight (1908) found in the case of carnations that one part of ethylene to 40,000 parts of air killed young buds and prevented the opening of buds already showing petals, while a concentration as low as one part to 2,000,000 caused closing of flowers already open. Using etiolated pea seedlings, Knight and Crocker (1913) found that one part of ethylene to 10,000,000 parts of air prevented elongation of the epicotyl, while four parts to 10,000,000 of air gave the triple response of Neljubow. E. M. Harvey (1915), working in the same laboratory, found that castor bean leaves give a definite nastic response in a concentration of one part of ethylene to 10,000,000 parts of air. The work of Crocker and Knight also indicated that the amount of ethylene present in illuminating gas probably determines the toxic limit of that gas. The responses of carnations, etiolated pea seedlings, and castor bean leaves to ethylene were far more delicate than any possible chemical test for this substance.

Richards and MacDougal (1904) observed no such delicate response in the case of seedlings of *Vicia Faba*, *Zea Mays*, and *Helianthus annuus*, growing in atmospheres composed of about eighty per cent illuminating gas and twenty per cent oxygen. The chief effects noted by them for these high concentrations of illuminating gas were decreased rate of growth, poor differentiation of tissues, retardation of chlorophyll formation, and increase in size of the cells of the cortical parenchyma. These same effects were noted when carbon monoxide was substituted for illuminating gas.

Wehmer (1917) reported water cress as fresh and green after being grown for twenty days in an atmosphere containing twenty per cent of illuminating gas.

Leaf fall, stimulation of root development, and formation of abnormal tissues seem to be common phenomena exhibited by plants as responses to illuminating gas. Thus Kny (1871), Späth and Meyer (1873), Wehmer (1900), Wilcox (1911), and others, as mentioned above, have reported leaf fall for various trees, shrubs, and herbs as a result of accidental or experimental gas poisoning. In some cases leaves turned yellow before falling, while in others there seemed to be only a stimulation of the abscission layer, resulting in the cutting off of the leaf. One of the best examples of defoliation from gas exposure is that given by Wilcox (1911), in which he described the effects of a leaky gas main upon the plants in a commercial greenhouse. The defoliation of the roses was complete, while the effect upon *Coleus* was only slightly less pronounced.

Stimulation of root development was reported by Boehm (1873) for twigs of willow kept in gas-saturated water. Richards and MacDougal (1904) noted a similar effect upon seedlings. Stone (1913) even attempted to use illuminating gas to facilitate the rooting of woody cuttings. He also found a forcing of foliage development, gassed twigs putting out leaves several days before the controls. This is in agreement with the general knowledge of the stimulating effects of low concentrations of poisons upon plants. Some, such as ether and chloroform, have actually been found of value to florists as forcing agents.

A very interesting aspect of the subject is the development of abnormal tissue in plants exposed to ethylene or illuminating gas. There is, however, little agreement as to the tissues affected

and the nature of the proliferations. This lack of agreement may be due to differences in the experimental materials or the experimental methods used by the various workers. Differences in the seasons of the year in which the experiments were made may be of significance.

The development of abnormal tissue is most striking in woody plants, but it is seen also in herbaceous plants and seedlings. Neljubow (1901), in describing the triple response of etiolated pea seedlings, gave increased growth in thickness as one of the effects of ethylene. This response was also observed by Crocker and Knight (1908). Richards and MacDougal (1904) reported a doubling in the size of the cells of the cortical parenchyma in seedlings grown in illuminating gas, as well as a failure in the normal differentiation of the tissues. Substances other than illuminating gas and ethylene have been found to cause proliferations in plants. Grottian (1909) reported that anaesthetics, especially chloroform, caused swellings in roots. Němec (1904) noted similar effects of ether, chloral, benzine, and alcohol vapors.

Woody materials, of both roots and stems, have been found to give rise to proliferations or intumescences under certain conditions. Stone (1913) has described an interesting case of abnormal tissue formation in *Populus deltoides*. The tree, some eight inches in diameter, had been poisoned by gas in the soil and showed on the side of the trunk on which absorption had occurred lesions from ½ to 1½ feet long, extending upwards from the level of the soil. At the base of these lesions were pads of delicate parenchymatous tissue from ½ to 1½ inches thick that had apparently arisen from the true cambium. The phloem and xylem remained normal in histological appearance. He also described proliferation and swelling for lenticels of willow shoots that had been kept in gas-saturated water.

Gatin (1912), in studying the effect of tarred roads on the adjacent vegetation, found a stimulation of cork formation at the phellogen and a disappearance of the endodermis. The affected trees developed a very small amount of woody tissue, but the phloem remained normal. The cells of the cortical parenchyma were normal in size and form. Analyses of the tarry materials showed the presence of many unsaturated compounds, and he thought that perhaps they were the toxic substances.

Harvey and Rose (1915) tested the effects of illuminating gas and ethylene on root systems. Seedlings of *Catalpa speciosa*, and *Ailanthus*, after exposures of from five to twenty-one days to various concentrations of ethylene or illuminating gas, developed cortical swellings, but similar tests with *Gleditsia* gave no swellings. Both hypertrophy of cortical cells and rapid proliferation of the phellogen layer were said to be involved. When strong streams of gas were passed through soil about the roots of larger potted plants, death ensued in a few days. But when only a small current (about 40 cc. daily) was used, stimulation occurred, resulting in the formation of abnormal cortical tissue on the roots. These abnormalities were reported for *Hibiscus*, *Syringa*, *Diervilla*, *Ricinus*, *Ulmus*, croton and pear.

Doubt (1917) extended the experiments further, using two- or three-year-old trees and allowing the gas of various concentrations to flow around the roots continuously for from fifteen to ninety days. Her results are similar to those of Harvey and Rose. All proliferated tissue was found to be external to the vascular cylinder of the roots, but whether it arose from the cortex or the pericycle was not determined.

Wehmer (1918) obtained results which indicate that trees vary in their sensitiveness to gas poisoning with the time of year. He passed continuous streams of illuminating gas through soil in which were rooted three- to seven-year-old deciduous and evergreen trees. In the spring they were killed in a short time, as was the case with herbaceous plants at all times. In late summer and early fall the trees were defoliated. In the dormant winter condition the trees gave no apparent response. He mentions no abnormal tissue formations such as those found by Stone, Gatin, Harvey and Rose, and others.

Woffenden and Priestley (1924) exposed elder plants to constant streams of illuminating gas by encasing the stems in tubes and passing gas through the tubes. The reaction obtained was found to vary with the stage of development of the stems. Young apical regions were killed in a few hours, older internodes directly below showed intumescences in the lenticels. Portions lower than the fifth node became discolored and swollen. The experiments, begun on July 1, were discontinued on August 25, because after that time no reaction could be obtained. The cessation of the capacity to react is attributed to the completion

of the cork layer which occurred about this time. They attribute the increased rate of cell division to increased permeability of the cells of the phloem and cortex, and suggest that the unsaturated linkages of the gas compounds unite with those of the unsaturated fatty acids in the cells, and thus give rise to more diffusible compounds, which spread outward and leave the whole cortex more permeable to water and nutrient materials. They sum up the work on woody stems in the following manner:

> Earlier work upon the effect of coal gas upon plants does not always provide sufficient anatomical data for exact comparisons, but there can be little doubt that where these observers record effects upon the bark of woody plants their results admit of explanation in the light of the experiments described above, and in every case are due to cells arising from the cork phellogen originally proliferating and failing to suberize.

Harvey and Rose (1915) mention hypertrophy of cortical cells and activity of the phellogen as being responsible for the abnormal tissue. They give figures for *Catalpa*, *Ailanthus*, *Syringa* and *Hibiscus*, the first three of which indicate cortical division unless the phellogen cuts off cells internally rather than externally. For *Hibiscus*, on the other hand, the figure shows the proliferation to be definitely phellogenic in nature. They fail to distinguish between the two types of figures. Doubt (1917) says all proliferation is external to the vascular cylinder, but does not know whether it arises in the pericycle or cortex. She makes no mention of the phellogen. In the case of the proliferated pads of parenchymatous tissue reported by Stone (1913) for *Populus deltoides*, it is physically impossible for it to have arisen from the cork cambium since phellogen could not give rise to tissue lying between xylem and phloem. From the results reported in the literature it is therefore evident that although the phellogen in many cases appears to be the reacting tissue, nevertheless in other cases proliferation may occur in other tissues.

MATERIALS AND METHOD OF EXPERIMENTATION

My studies have so far been confined entirely to woody stems and have extended over two consecutive years, chiefly in the fall and winter period from September to March. The material for the experiments consisted of branches from 1 to 1.5 cm. in diameter cut from trees or shrubs growing out of doors. The

shoots were then cut into short lengths (10 to 15 cm. long) and the latter were grouped into sets so that each set contained a graded series of sizes from 1.5 cm. in diameter down to slender terminal twigs. These sets were placed in belljars with the basal end of each piece in moist sand as if for rooting experiments. A measured volume of ethylene was liberated in each jar and left for twenty-four to forty-eight hours, after which the jars were opened for twenty-four hours to allow all the ethylene gas to escape. The jars were then left closed except when observations were being made. A similar set of control twigs, receiving identical treatment except for the absence of the ethylene, was provided with each experiment.

The reactions observed in this study are those which may be obtained with woody twigs collected in the season of the year during which physiological processes are the least active. Rather low concentrations of ethylene for a single rather short period were used. After the exposure to the gas, the twigs remained in a water-saturated atmosphere, free from the gas. It was found that the reaction might take place within a few days after the exposure to the gas, or it might require several weeks.

GENERAL REACTIONS

Twigs in the controls showed only slight responses to the higher temperatures and humidity of the environment provided. In one experiment which may be taken as typical of many others, enlargement of the lenticels occurred in only three species out of fifty within three weeks after the experiment was begun. Aerial roots appeared in four species (*Populus candicans*, *Salix purpurea*, *S. pentandra*, and *S. vitellina*). Only a few species were found to leaf out in experiments made during fall and early winter, but during late winter and early spring many species developed complete series of leaves. Callus formation at the basal end of the twigs occurred in more than fifty per cent of the species tested. Among the twenty-eight that formed callus, eleven showed approximately equal development of callus on both the morphologically apical and morphologically basal ends, while the remaining seventeen formed it only on the basal ends.

Many of the responses reported for plants in general have been found to occur in woody stems following the gas treatment outlined above. Apple twigs which were tested early in the

autumn before the leaves had fallen showed defoliation within two or three days, the leaves remaining green. Stimulation of root formation resulted in many plants but was more pronounced at certain seasons than at others. Proliferation of cells in the lenticels occurred in *Sambucus*, *Syringa*, *Catalpa*, and others.

Forcing of buds was observed in almost every species tested in this study. Buds may be forced at almost any season. A rather interesting relation has been noted in this connection. In the early winter the resulting leaves are more or less abnormal, appearing as if burned on the edges, while later (usually early in February) the buds may produce healthy leaves.

Although ethylene has been used to cause the disappearance of chlorophyll from certain plant parts (such as celery leaves and tomato fruits), it appears to produce no such effect on the chlorophyll of the chlorenchyma of woody stems. I have frequently observed cases in which the cork was disrupted and the green layers below were exposed, but in no case have I observed an apparent decrease in the chlorophyll content of the cells. This is true both for experiments made in darkness and in light.

THE FORMATION OF INTUMESCENCES IN APPLE TWIGS

It is well known that hypertrophy may occur in the tissues of stems and roots when they have been exposed to stimulating agents. *Gymnosporangium* galls, crown galls, etc., arise from parasitic stimulation of the host tissues. In the former case, Stewart (1915) stated that it is proliferation of an axillary bud that gives rise to the gall. Smith (1917) was able to simulate closely crown gall formation on *Ricinus communis* by injecting various substances into the plant, and concluded from this evidence that crown galls are formed in response to chemical stimuli by the substances which are by-products of the life activities of *Bacterium tumefaciens*. Hahn, Hartley, and Rhoads (1921) report hypertrophied lenticels on the roots of conifers that had been grown in excessively moist soil. Plants growing in bog water are reported commonly as having abnormal roots. The work of Harvey and Rose (1915) and Doubt (1917) has shown clearly that many woody plants will develop proliferated cells in the cortical region if illuminating gas or ethylene is passed through the soil around the roots.

Apple stems, when in the most sensitive condition, may show,

as a result of ethylene stimulation, intumescences in their buds, at either their basal or apical ends, or in their internodal regions. Of the three responses, that in the buds is the most striking and so far as I can find has never been reported for any plant. The reaction appears first as a transverse breaking of the epidermis and cork layers on each side of the bud base (FIG. 3). The actual time required for the appearance of the reaction is in some cases as low as forty-eight hours, while in others as long as six days may intervene between stimulus and reaction. In general about four days is required. Proliferation of the cells of the bud and bud base may proceed very rapidly and within three or four days the entire resting buds, containing the leaf primordia for the following spring, may be completely destroyed. The tissues of the buds, some of which already are highly differentiated, apparently revert to an embryonic condition and the resulting mass of living cells has a loose granular appearance. This reaction in the buds extends to the xylem of the stem. Externally the buds at this time present an appearance very suggestive of popped corn (FIG. 1). The cork layer, bud scales, etc., are curled back, while the soft, almost white, proliferated cells protrude. A slight jar often suffices to cause a mass of the proliferated tissue to fall away from the stem. Not infrequently the same kind of reaction occurs in the region of bud-scale scars which mark the position of the terminal bud produced at the end of some previous year's growth (FIG. 4).

Terminal swellings usually begin before the bud reactions are complete; that is, from five to eight days after exposure to the gas. Terminal swellings are very common on cut ends of twigs, but in some cases appear also on the uncut ends of short lateral branches. They first become evident by a breaking of the epidermis near the tip of the twig (FIG. 5), due to an enlargment of the tissue beneath. For several days they may remain as slight swellings in the bark, but within a week they may become very pronounced, in many cases increasing by 6 or 8 mm. the normal diameter of the twig. This occurs even in the case of twigs only 4 mm. in normal diameter (FIGS. 6, 7). As may be seen in the figures, the line between normal and swollen tissue is very sharp.

The internodal swellings occur later than the terminal swellings, appearing in from nine to twelve days after exposure

to the gas and reaching their maximum development five to ten days later. The internodal swellings seem to be little correlated with either the bud or the terminal reactions, since they often occur when neither of the others is present (FIGS. 10, 11). The exact depth in the bark to which the terminal and internodal swellings penetrate has not been definitely determined, but there appears to be in many cases a combined hypertrophy and hyperplasia of both phloem and cortex. This phase of the problem, as well as the cytological data relating to the behavior of the cell structures, is to be discussed in my full report.

Although bud destruction, terminal swelling, and internodal swelling constitute the typical series of reactions when the twigs are most responsive, intumescences of the buds may not occur, while terminal and internodal swellings follow in their regular sequence.

EFFECT OF ENVIRONMENTAL CONDITIONS ON THE REACTIONS OF APPLE TWIGS

Experiments on the relationship of external conditions to the response above described have given some interesting suggestions. For the present I shall give only a summary of my experiments, leaving until later the detailed statistical treatment of the data. The statements are, in every case, based upon concordant results from several experiments.

Concentration and presentation time. Concentrations greater than one part of ethylene to 4,000 parts of air showed little or no increased intensity of reaction. Identical responses in all three regions were obtained with a series of concentrations ranging from as low as one part of ethylene to 4,000 parts of air to as high as one part of ethylene to seven of air. Exposures of one, two, and ten days gave identical results in buds and terminal intumescences; with the longer periods there was a doubtful increase in the degree of internodal swelling. Further experiments are being made with low concentrations and shorter presentation times.

Temperature. All experiments were made in greenhouses with a mean temperature of about 21° C. and a daily range of from 10 to 15° C. I have collected twigs at a widely varying range of temperatures. Material was collected before frost

in the fall as well as at -20° F. in December. Pronounced intumescences were obtained in buds, ends of twigs, and internodes in material collected at all these temperatures.

Wounding as an aid to penetration by the gas. Readiness of penetration of the gas into a tissue might affect the time and degree of response of the tissue, all other conditions being constant. Experiments designed to test this factor, however, showed negative results in all cases. Scraping, notching, or slitting of the bark of the twigs before they were exposed to the gas had no apparent influence on the responses. Buds which were covered with moist sand, owing to their position near the lower end of the stems, reacted as did those above in the air. Inverting the twigs brought out an interesting result. The buds and internodes react as do those of twigs in normal position; the morphologically apical end now being downward does not react, while the morphologically basal end which is above does react. The terminal swelling may, therefore, be obtained from either the morphologically apical or basal end according to its position.

Species of plants which will react. Forty-seven species of woody plants were tested, and they have shown that it is rather rare for intumescences to be formed in all three regions, buds, internodes, and apices of the same plant. A cultivated crab (*Pyrus Malus*), Transparent apple (*P. Malus*), *P. ioensis*, and *Ginkgo biloba* each gave responses in the three loci. Certain other kinds of stems showed intumescences in only one or two of the regions: *Populus deltoides* (FIG. 2), *P. tremuloides*, and *Salix longifolia* gave responses in the buds and also gave internodal swellings, but no terminal swellings. *Syringa*, *Forsythia*, *Prunus virginiana*, and others gave internodal swellings, but no bud response or terminal swellings. A relatively large number, sixteen in all, including *Sambucus canadensis*, *Acer Negundo*, *Juglans cinerea*, etc., showed a stimulation of the lenticels, but failed to show any response in buds, apices, or internodes. Approximately fifty per cent of the species tested showed no visible response in the form of intumescences when treated with ethylene gas.

EFFECT OF ETHYLENE UPON CALLUS FORMATION

In the course of my experiments on the effect of ethylene upon stems it was observed that there was an apparent inhibition

of callus formation, though in general the effect of ethylene on twigs seems to be to stimulate cell division in some region or other. Experiments showed conclusively, however, that ethylene may inhibit the development of callus. Twigs from forty-seven species of trees and shrubs growing out of doors were cut on November 6 and placed in moist chambers. Twenty-eight had developed callus by December 14. Eleven of these species without gas treatment showed what might be called good callus, while twigs of the same kind treated for forty-eight hours with concentrations of 1–400 and 1–1600 showed no callus at all. In fourteen cases some callus was present on the treated twigs, generally in the lower concentration, but the callus on the untreated twigs was better developed. In the three remaining species the callus was apparently equally developed on both treated and untreated twigs; but in no case was it better developed on the treated twigs than on those not treated. A repetition of the experiment, using twigs cut from the same trees on January 26, showed on February 7 results concordant with those of the first experiment.

GENERAL DISCUSSION

I have not determined just how and where these intumescences arise in the tissues of the apple. The pads of parenchymatous tissue described by Stone (1913) for *Populus deltoides* seem from both the text and figures to be cambial in nature. Harvey and Rose (1915) and Doubt (1917) are very specific in stating that the swelling was external to the vascular cylinder in the woody plants studied by them. The apple intumescences in the buds, in the apices, and in the internodes seem to involve proliferation of tissues within the vascular cylinder as well as external to it. The bud intumescences are so striking in appearance that had any of the earlier students of gas effects seen them their description could leave no doubt as to the identity of the reactions.

The lowness of the concentration and the shortness of the presentation time which were found sufficient to bring about a response raise some interesting questions. The lowest concentration and the shortest presentation time so far used were one part of ethylene to 5,000 parts of air for twenty-four hours and one part of ethylene to 400 parts of air for two hours; and under

these conditions definite intumescences in the three loci developed. Harvey and Rose (1915) and Doubt (1917) used long exposures to gas in their experiments on woody plants, none being for less than five days and some being for as long as three months. It would be interesting to know if similar responses would have resulted from presentation times of twenty-four hours or less. This would seem probable if the reactions are analogous to those I have found in apple twigs.

As previously mentioned, Denny, Harvey, and others have caused chlorophyll to disappear from certain plant parts by exposing them to ethylene. The chlorophyll content of the chlorenchyma of woody stems, however, was not visibly diminished by ethylene treatment, under the conditions of the present experiments. FIGURES 6 and 7 show two terminal swellings on Transparent apple twigs and in each case the cork layer has peeled back, exposing the chlorenchyma layer. In FIGURE 7 the chlorenchyma has split, exposing the white cortical and phloem tissues beneath. The dark shade of the chlorenchyma indicates that chlorophyll is still present. It should be pointed out in this connection that the disappearance of chlorophyll in lemon fruits and celery stalks is merely hastened by exposure to ethylene gas.

The apparent inhibition of callus formation by ethylene is exceptional, since many of the responses of plants to gas seem to be definite cases of stimulation of cell division. Experiments in which concentrations of one part of ethylene to 400 or 1600 parts of air were used showed inhibition for at least eighty per cent of the species that normally formed callus in the control. In many cases callus inhibition was the only evidence of injury that the twigs exhibited. Often, however, callus inhibition was found associated with another response to ethylene, the development of intumescences in the lenticels. FIGURES 12 and 13 show this association for *Sambucus* (FIGURE 12 shows control twigs, while FIGURE 13 shows the treated). Further experiments are in progress, testing lower concentrations and shorter presentation times, in an attempt to find a concentration or presentation time that may stimulate callus formation.

SUMMARY

Experiments on woody twigs conducted for two consecutive years, chiefly from September to March, showed many cases of intumescence formation in response to ethylene stimulation. The reactions were obtained by exposing cut twigs of various trees and shrubs to a known concentration of ethylene for a single period (from two to 48 hours), after which the twigs were kept in a saturated atmosphere free from the gas.

Different species varied greatly in their response to ethylene stimulation. The most pronounced type of intumescence, involving the destruction of buds, swelling of the apices and internodes, was obtained in *Pyrus Malus* (varieties Transparent apple and cultivated crab), *P. ioensis*, and *Ginkgo biloba*.

Concentrations greater than one part of ethylene to 4,000 parts of air showed little or no increased intensity of reaction. Identical responses occurred in concentrations ranging from 1–7 and 1–4,000.

Although a presentation time of forty-eight hours was used in most of the experiments, a presentation time of two hours gave a response.

Ethylene had no apparent effect on the chlorophyll in the chlorenchyma of woody stems.

At least eighty per cent of the twigs which normally formed callus in the controls showed an inhibition of callus formation after an exposure to a low concentration of ethylene gas.

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Description of plates 14 and 15

Plate 14

Fig. 1. Buds of *Pyrus Malus*, var. Transparent (× 1.5), five days after exposure to ethylene. Buds have formed intumescences; lenticels are normal.

Fig. 2. Twig of *Populus deltoides* (× 2) six days after exposure. Buds are raised upon a pad of proliferated tissue, which is characteristic of poplar and willow.

Fig. 3. Side view of reacting Transparent apple bud (× 4). Reaction is incomplete.

Fig. 4. Intumescence in a terminal bud scar (× 2) marking the end of a previous year's growth (Transparent apple).

Fig. 5. Transparent apple twig (× 2) ten days after exposure, showing completed intumescence in a bud and the beginning of a terminal swelling.

Fig. 6. Same twig as shown in fig. 5 but one week later, showing completed terminal swelling. Lesions in the chlorenchyma layer expose the white cortical tissue beneath.

Fig. 7. Transparent apple twig (× 2) ten days after exposure. Note the splitting of the cork and chlorenchyma layers, exposing the deeper tissues below. The contrast of light and dark indicate that chlorophyll is still present.

Plate 15

Fig. 8. Transparent apple twig (× 2) with terminal intumescence and slight indications of internodal intumescence ten days after exposure to ethylene. Note the intumescence in the terminal bud on the small lateral branch.

Fig. 9. Same twig as shown in fig. 8 one week later, showing intumescences in the terminal bud, apex, and internode of the same twig.

Fig. 10. Twig of *Pyrus ioensis* (× 1) showing normal internode.

Fig. 11. Twig of *Pyrus ioensis* (× 1) showing internodal swelling, but normal bud and apex.

Fig. 12. *Sambucus* twigs (× 1) from control, nineteen days after the experiment was begun. Good callus has developed and lenticels are normal.

Fig. 13. *Sambucus* twigs (× 1) nineteen days after exposure to the ethylene. The lenticels are enlarged and no callus has developed.

Fig. 14. Enlarged lenticels of *Sambucus* from ethylene jar.

Stone, G. E. 1913. Effects of illuminating gas on vegetation. Ann. Rep. Mass. Agr. Exp. Sta. **25:** 45–60. *f. 1–3.*

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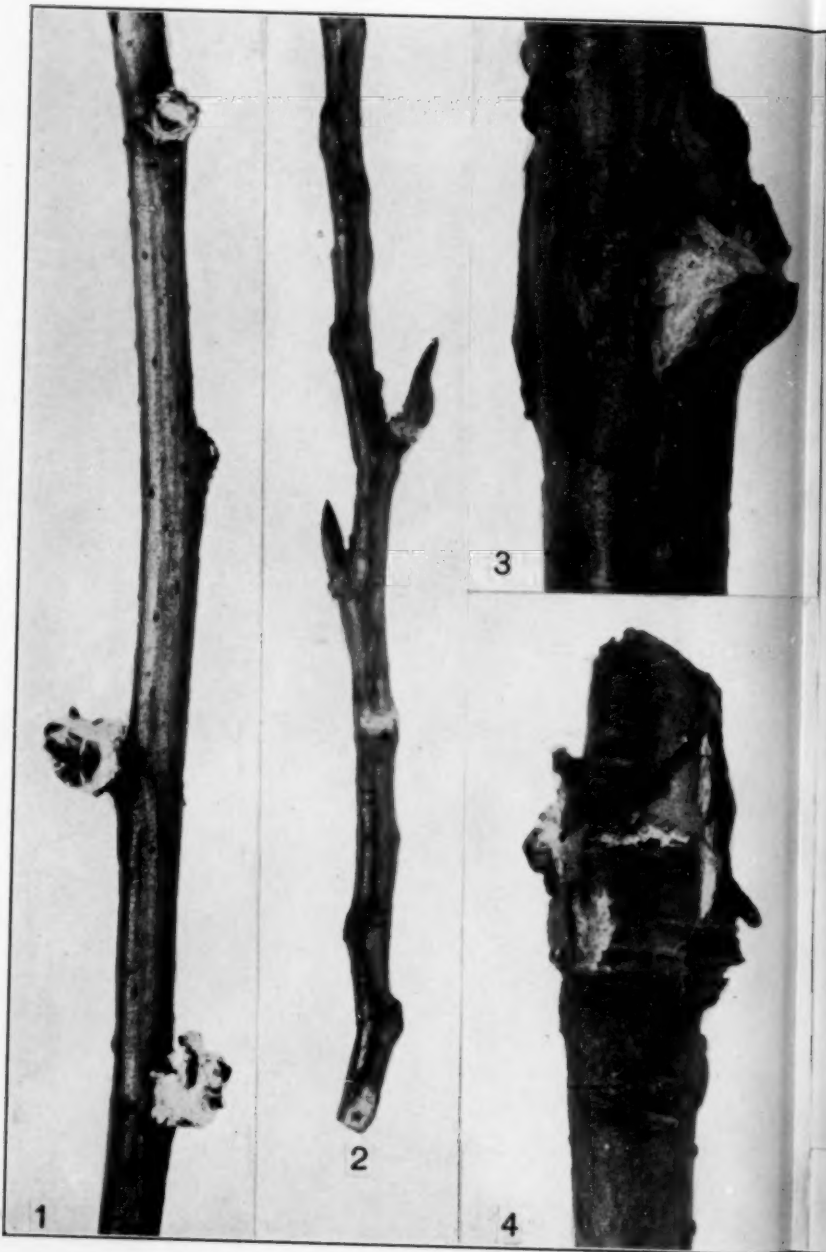
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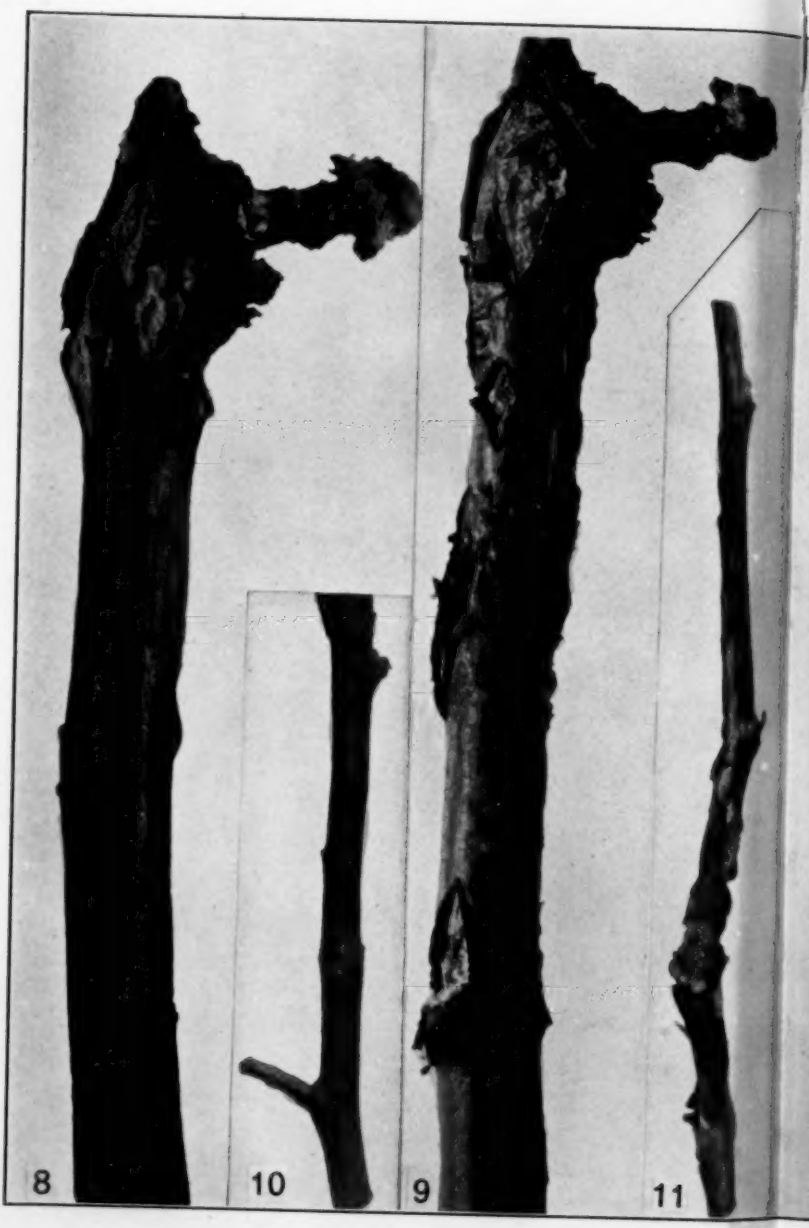
NTUMESCENCES ON TWIGS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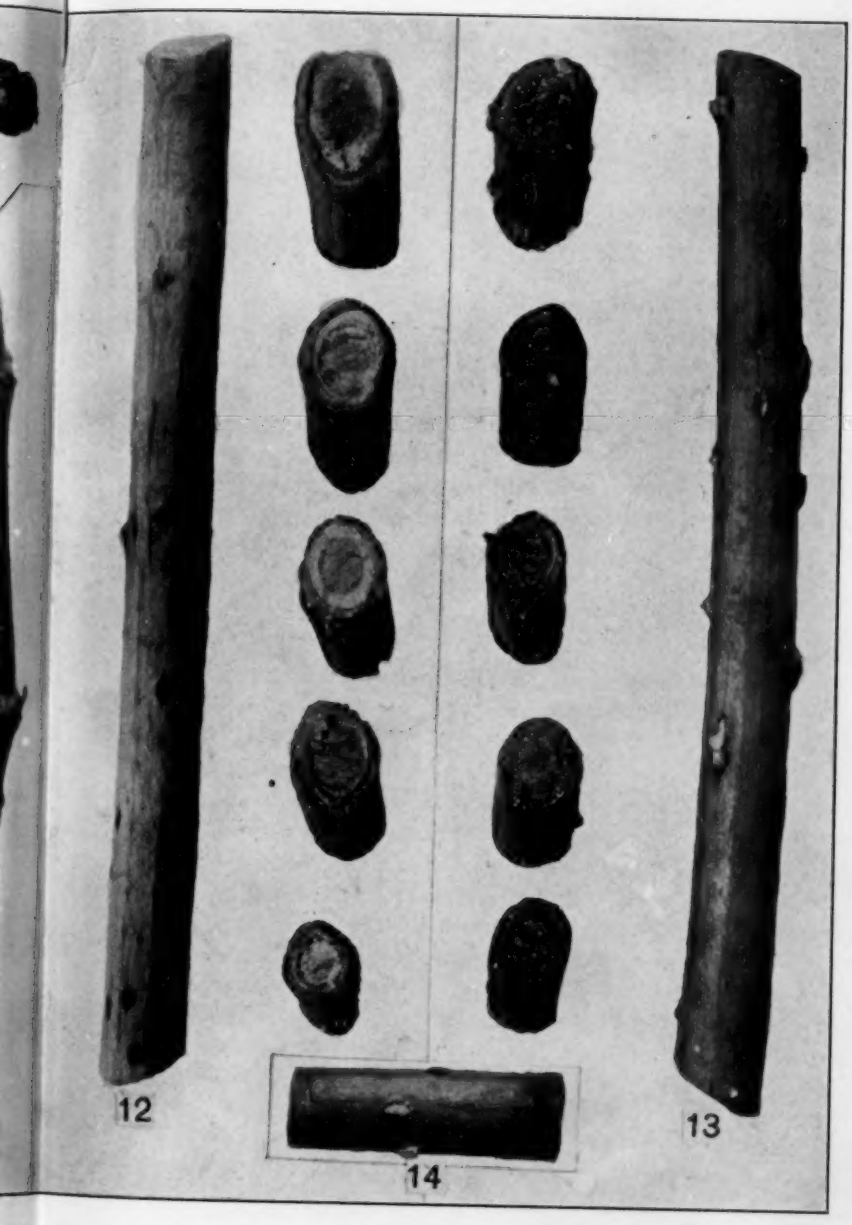

UMESCENCES ON TWIGS

UNIV OF MICH.

Notes on the flora of the Miocene of the Tesla region, California

FLORA MURRAY SCOTT

(WITH PLATE 16)

From the upper San Pablo (Miocene) of the Tesla region, Alameda County, during the summer of 1922, a small collection of fossil leaves was obtained; the specimens are deposited in the collection of Stanford University. The impressions are well preserved in fine grained sandstones and calcareous sandstones; they are occasionally partially carbonized. They represent the detritus of a temperate, well watered area with a rich and varied growth of broad-leaved trees.

The genera identified, besides one monocotyledonous specimen, are distributed through the following dicotyledonous families: Salicaceae, Fagaceae, Lauraceae, Platanaceae, Rosaceae, Aceraceae, Cornaceae, and Oleaceae.

DETAILED DESCRIPTION

The Tesla flora comprises 18 genera, of which 13 species have been indentified. The specimens will now be discussed in detail.

1. A monocotyledonous species. A small, but recognizable, fragment of a monocotyledonous leaf of the type of *Typha*.

2. POPULUS ALBA L. (?) Two leaf fragments of the genus *Populus* do not appear to be referable to any fossil species so far described from the Pacific Coast. The broad apex and widely crenate margin of the leaves are in marked contrast to the narrow or acuminate apex, and closely crenate or almost dentate margin of the majority of fossil and present day forms. These characters, however, are diagnostic of the European species *Populus alba*. The only fossil species which the specimens in question at all resemble is *Populus polymorpha* Newb., described by Newberry from Bridge Creek, Oregon. Two fragments, however, hardly constitute sufficient foundation on which to base a theory of the former distribution of *Populus alba*, nor considering the variability of leaves, is it advisable to attempt to delimit a new fossil species.

3. POPULUS sp. Another leaf fragment is referable to the genus *Populus*, in finely serrate margin, venation, and broadly ovate shape, showing a close resemblance to such a type as *Populus tremuloides* Michx.

4. SALIX sp. The genus *Salix* is represented by one small and perfect leaf and a number of fragments.

5. CORYLUS sp. One specimen shows the basal part of the leaf with the unmistakable form and venation of this genus.

6. ALNUS RHOMBIFOLIA Nutt. From the numerous, but unfortunately incomplete fragments of *Alnus*, it is seen that, when entire, the leaves are 3–5 cm. broad, and 5–6 cm. in length. The venation is well preserved, the tertiaries being particularly distinct. The leaf margin is slightly doubly serrate.

Alnus corrallina Lesq. from Corral Hollow is described as follows (Lesquereux 5):

> Leaves oblong-ovate, thickish, rounded in narrowing to a short petiole, obtusely pointed, doubly denticulate; teeth short, acute, turned outside, glandulose; secondary nerves close, parallel, straight to the borders, branching in the upper part; nervailles distinct, close, simple, rarely branching, at right angles to the veins; catkins . . .

From this description and from the figures which accompany it, the present leaf impressions differ only in their slightly larger size, a point of minor importance in leaf identification.

Lesquereux remarks on the close affinity of *Alnus corrallina* with the present day *Alnus viridis* of the eastern states. On comparing the present fossils with herbarium material, it is seen that, from a consideration of leaf alone, it is impossible to distinguish between the Tesla leaf impressions and the following species: *A. viridis*, *A. rhombifolia* and *A. rugosa*. Age and habitat may accentuate certain variations in size, in leaf margin, and in the prominence of the tertiary veins. This last character is perhaps most constant in *A. rhombifolia* Nutt., one of the commonest alders of the Pacific Coast. In short, *Alnus rhombifolia* Nutt. appears to be identical with *Alnus corrallina* Lesq., and may therefore be regarded as dating back to Miocene.

7. QUERCUS KELLOGGII Newb. One fragment of a large oak leaf, 5.5 cm. broad and probably 12 cm. long when complete, resembles very closely the California Black Oak, *Q. Kelloggii*. *Quercus pseudo-lyrata* Lesq., a fossil species from the John Day

Basin, in form and in venation bears a striking resemblance both to the Tesla fossil in question, and to the present day leaf of *Q. Kelloggii*, a species which ranges from Oregon to San Diego.

8. QUERCUS CHRYSOLEPIS Lebm. Another species of *Quercus* is present in a small but perfect leaf, that is not to be distinguished from *Q. chrysolepis*, the present common Canyon Oak of the Pacific Coast.

9. CASTANEA sp. Two small fragments in venation and in leaf margin show a very close resemblance to the modern chestnuts, in particular to the species *Castanea pumila* (L.) Mill.

The family Lauraceae is important in the Tesla flora, being represented by no less than seven forms, belonging to the genera *Laurus*, *Persea*, *Umbellularia*, and *Cinnamomum*.

10. LAURUS GRANDIS Lesq.; 11. LAURUS PRINCEPS Heer; 12. LAURUS SP. The two fossil species recorded by Lesquereux (5) from Corral Hollow, *L. grandis* and *L. princeps*, are identifiable in a few large and well preserved fragments. A single leaf fragment, elliptical in outline, coriaceous, and pinnately veined, may also be referred to the genus *Laurus*.

The entire absence of this genus from North America today is interesting in view of its previous very wide distribution. Of present day forms the species *Asimina triloba* Dunal shows the closest resemblance in venation to the fossil species of the Lauraceae.

13. PERSEA BORBONIA Spreng., and 14. PERSEA ALPIGENA Spreng. (FIG. 2) *Persea*, a tropical and subtropical genus of the American continent, fairly common in the Southeastern States, at one time appears to have flourished on the Pacific Coast. Numerous leaves in the Tesla formation are not to be distinguished from present species of the former region, viz., *P. Borbonia* Spreng. and *P. alpigena* Spreng.

15. UMBELLULARIA CALIFORNICA Nutt. The sole and by no means unimportant member of the family Lauraceae on the Pacific Coast today, *Umbellularia californica* Nutt., is recognized in one fragmentary fossil. While bearing a certain resemblance, especially in the form of the leaf, to *Salix lasiolepis* Benth., the more leathery texture, and in particular the fine and very distinct reticulation, of *Umbellularia* appear to be diagnostic.

16. CINNAMOMUM sp. The tri-nervation of one widely ovate-elliptical fossil leaf refers it to the genus *Cinnamomum*.

17. PLATANUS DISSECTA Lesq. A great part of the leaves of the Tesla collection appear to belong to the genus *Platanus*. The leaves of *Platanus dissecta*, first described by Lesquereux from Corral Hollow, are extraordinarily well preserved, though unfortunately they are in all cases incomplete. From the very numerous fragments, however, a fairly complete reconstruction is possible.

Along with the leaves are found many well preserved pieces of rugose bark (FIG. 4). This appears to be identical with the bark of the present day form, *Platanus racemosa* Nutt. In leaf also, *Platanus dissecta* very closely resembles the modern species. A point of difference is seen in the leaf margin. This, in *Platanus racemosa*, is usually entire. In one specimen from Colusa County, California, however, the margin is toothed, differing in no wise from the margin of the fossil species.

18. PRUNUS DEMISSA Walp. (FIG. 1). Very numerous leaves, both complete and fragmentary, of the genus *Prunus*, are not referable to any Pacific fossil species, from all of which they appear to differ in the nature of the secondary veins. These, in the majority of fossils, are inconspicuous, and disappear towards the leaf margin. In the Tesla specimens, however, they are well marked, somewhat irregular, and arcuate towards the margin; the venation is in brief similar to that seen in the present day *Prunus demissa* Walp. The leaf margin is not always distinguishable in the fossils, but a few specimens show a minute serration like that of the present form.

19. ACER sp. Two specimens, at first included along with the Platanaceae, may possibly represent the genus *Acer*. They are unfortunately incomplete, but show a very close resemblance to the fossil species *Acer Osmonti* Knowlton, and also to the present day sugar maple, *A. saccharum* Marshall.

20. CORNUS NUTTALLII Aud. One leaf fragment resembles very closely the present Pacific dogwood, *Cornus Nuttallii*.

21. FRAXINUS OREGONA Nutt. (FIG. 3). Ash leaflets, identical with the present day species *Fraxinus oregona*, are abundant and perfectly preserved. In one specimen the leaflets are seen to be nearly sessile.

Fraxinus oregona differs in certain characteristics from the

Pacific Coast fossils already described from the Clarno formation, viz. *Fraxinus oregonensis* Knowlton and Cockerell, and *F. denticulata* Heer. The Alaskan form *Fraxinus heerendensis*, while closely resembling the Tesla species, is distinguished by the toothed leaf-margin.

22. CHRYSOPHYLLUM sp. A fragmentary leaf, conspicuous in the right angled origin of the closely set parallel secondary veins, bears a striking resemblance to the genus *Chrysophyllum* and in particular to the Porto Rican species *Chrysophyllum Cainito* L. *Chrysophyllum* today is a tropical and subtropical genus, being represented by a few species only in the Southeastern States. It does not occur on the Pacific Coast.

COMPARISON WITH OTHER FLORAS

The two main Miocene floras of the Pacific Coast with which the Tesla flora may be compared are: (1) flora of the John Day Basin (Mascall) Oregon; (2) flora of the Auriferous Gravels (Knowlton 2, 3). On comparing the Tesla fossil list with the former, it is seen that, with the exception of *Laurus grandis* Lesq., *Laurus princeps* Heer, *Platanus dissecta* Lesq., the Tesla species are absent from the Mascall formation; nor are the following genera represented:—*Umbellularia*, *Cinnamomum*, *Fraxinus*, *Chrysophyllum*. Similarly from the Auriferous Gravels the following fossil species occurring in the Tesla beds are reported:—*Laurus grandis* Lesq., *Laurus princeps* Heer., *Platanus dissecta* Lesq.; while the genera *Alnus*, *Umbellularia*, *Cinnamomum*, and *Chrysophyllum* do not occur.

On the other hand the fossil species of *Aralia*, *Artocarpus*, *Ficus*, *Liquidambar*, and *Magnolia*, common to the Mascall formation, and to the Auriferous Gravels, do not appear in the Tesla formation. In short, in genera and species the Tesla flora is strikingly modern.

The distribution of the modern forms is interesting. The majority are common on the Pacific Coast. Three species, however, do not occur west of the Continental Divide, viz., *Populus alba* L., *Persea Borbonia* Spreng., and *P. alpigena* Spreng. *Populus alba* L. is today a European form. The identification of this species here rests, as already stated, on somewhat scanty material, and therefore, pending further fossil evidence, it may temporarily be disregarded. The genus *Persea* today ranges

along the Coastal belt from the Southeastern States as far south as Brazil and Chile, i. e., it is a tropical and subtropical genus.

The presence of the genera *Persea*, *Chrysophyllum*, and *Cinnamomum*, which have survived from the definitely subtropical Eocene, may be taken to indicate that, during the time of the Tesla deposition, conditions slightly warmer than those of the present day obtained; while the absence of *Aralia*, *Artocarpus*, *Ficus*, *Liquidambar*, and *Magnolia*, of the Mascall formation and of the Auriferous Gravels, genera today typical of subtropical regions, is significant of the changing climate.

GENERAL SUMMARY

The preponderance of present day species in an Upper Miocene flora appears, in comparison with other floras of the same age, to be somewhat unusual. When, in the process of identification, herbarium material is examined, the extraordinary variability of leaf forms is at once apparent. A complete and continuous series of variations can be traced in such characters as the following: the size and shape of the leaf, the angle of the apex, the nature of the leaf margin. One thing only remains relatively constant, and that is the venation. The ultimate branching of the veins, determining the leaf areolation, may also be included in this category, but this finer reticulation is not always preserved in the fossil state. Even in these last characters, however, modifications constantly occur. Age and habitat may not only obliterate distinctions but may impose thereon resemblances entirely illusory. From a leaf alone accurate specific determination appears to be impossible. This fundamental capacity for leaf-variation must be considered when dealing with a fossil flora. For this reason, it appeared to be impossible to delimit the Tesla fossils from the present day species. In all cases the leaf impressions showed a closer resemblance to modern forms, as represented in herbarium material, than to any fossil species hitherto described.

Should this same fact emerge from a consideration of other fossil floras, then the present ideas on the longevity of plant species will require to be somewhat revised.

CONCLUSIONS

1. The Tesla flora is broad-leaved in character, the flora of a moist and temperate region.

2. That the climate may have been slightly warmer than today at the time of Tesla deposition is suggested by the presence of the at least subtropical genera *Persea*, *Cinnamomum*, and *Chrysophyllum*.

3. The Tesla flora differs from other fossil floras of a like age in its exceedingly modern aspect.

4. Certain present day species may be regarded as dating back to upper Miocene.

5. The absence of the genus *Laurus* from the present day flora of North America is so far unexplained. The conditions which brought about its restriction and ultimate extinction are unknown. A general resemblance to the genus *Asimina* is noted.

6. The fossil species *Alnus corrallina* Lesq. does not appear to differ from the present *Alnus rhombifolia* Nutt.

7. Considering the wide range of fossil species the use of a fossil flora in detailed stratigraphy is therefore seen to be practically nil.

The main interest of the fossil plant record lies in evidence it affords regarding former climatic conditions, and in these so called "fossil" climates lies a key to the present day distribution of plants.

I have pleasure in expressing my thanks to Dr. J. P. Smith and Dr. LeRoy Abrams for their criticism and assistance in the identification of the above fossils.

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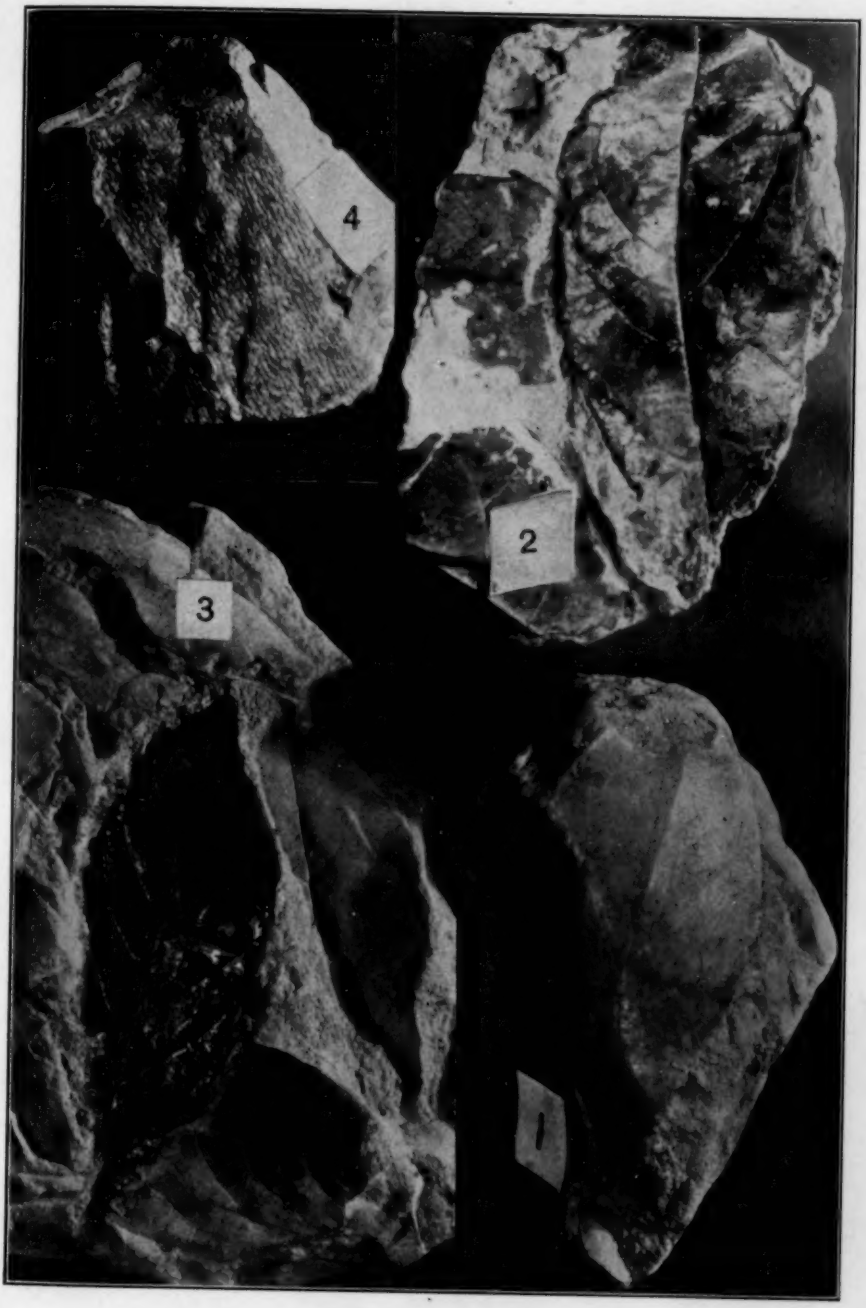
Explanation of plate 16

FIG. 1. PRUNUS DEMISSA Walp., leaf.
FIG. 2. PERSEA ALPIGENA Spreng., leaf.
FIG. 3. FRAXINUS OREGONA Nutt., leaf.
FIG. 4. PLATANUS RACEMOSA Nutt., bark.
All of the photographs are natural size.



SCOTT: TESLA REGION

UNIV. OF MICH.

Changes in plastids in variegated plants[1]

ILLO HEIN

(WITH PLATE 17)

If on the margins of the chlorotic areas of variegated leaves there is a sharply marked transition zone between cells with normal green plastids and those with no plastids or with colorless plastids, it would seem that there are two types of cells present, whose characters may be transmitted independently in heredity. If, on the other hand, the transition from colorless cells to those with normal green plastids is gradual, it suggests the origin of the modified area by the diffusion outward from a center of some toxic substance.

Baur (2) believes that there are essentially two types of variegation: a very common non-infectious type which is more or less transmissible through seed, such as occurs in *Pelargonium zonale;* and a rarer type which is not transmissible through seed and is decidedly infectious, such as occurs in *Abutilon Thompsoni*. Baur's work on the chimera has been well reviewed by Küster (13) and by Noack (18), and on infectious types by Shull (20) and by Clinton (7). As a result of numerous experiments with infectious chlorosis in species of *Abutilon* and other members of the mallow family, Baur has concluded that it is caused by a chlorophyll destroying toxin which is produced in the infected leaves.

That variegated abutilons could be produced only by grafting has been known to horticulturists for over 200 years, and the method was described by Morren (17) and by Lindemuth (15). Morren demonstrated that the variegated abutilons could not be propagated by seeds.

For the non-infectious type of chlorosis in *Pelargonium*, Baur (4, 5) finds that the plastids in the white cells uniformly contain little or no chlorophyll and are smaller than the plastids in the green cells, but he does not state whether or not there is an area of gradation between the two types. The white cells arise from pre-existing white cells, and green cells from pre-existing green

[1] Contributions from the Department of Botany of Columbia University, no. 346.

cells, the two kinds arranged in sharply defined layers or sectors, these producing the so-called chimeras. The various arrangements of the tissues in these types have been discussed by Baur (5) Noack (18) Küster (13) Chittenden (6) Stout (20) Correns (8) and Bateson (1).

As a possible working hypothesis for the origin of the chimeral type of variegation, Baur (4) suggests the possibility that a fertilized egg which originated from the union of a 'white' sex cell and a 'green' sex cell would have two kinds of plastids, green and white, and the vegetative segregation of these plastids in successive cell divisions might result in various distributions upon which would depend the appearance of the leaves.

There may be pure green, pure white, or sectorial chimeral leaves and sprouts. In all these, however, the transition from green to white should be abrupt. Noack (18, 19) points out that the chimeral, the pure white, or the pure green sprouts could not arise by direct cytoplasmic inheritance and the vegetative segregation of the two kinds of plastids, because of the relatively small number of plastids that can possibly be contributed by the male cell, and the very large number of plastids to be expected in the egg cell. In such crosses as he claims the hereditary factors must be found in the nucleus.

Data supporting the theory of the transmission of variegation by chromosomal genes have been reported by Chittenden (6). Anatomical studies of certain varieties of *Pelargonium zonale*—Freak of Nature, Caroline Schmidt, and Baur's B—and of *Hydrangea hortensis* show sharply differentiated white and green tissues; in the former there are no plastids and the change from white cells to green cells is abrupt. For these the assumption of distinctive factors for white and green may seem plausible. However in a variety of *Pelargonium*, Happy Thought, whose leaves are greenish yellow in the center surrounded by a green border, the sections do not show a sharp boundary between the yellow and green cells, but there is a region several cells thick in which gradations from pale yellow to green occur. The plastids are difficult to see in the pale areas and become greener in the area of transition. Chittenden states that this is not a periclinal chimera with white central tissue surrounded by peripheral green cells, but holds that, "Presumably this yellow tissue is due to heterozygosis with one or more bleaching factors, the recom-

binations of which with each other and with the normal green" give the results. It does not seem clear from Chittenden's account how factors for bleaching and factors for green give the intermediate stage in the marginal cells. The assumption of some substance diffusing radially from cell to cell and gradually bleaching the plastids seems simpler for such cases.

The condition of the plastids in the modified areas of many variegated plants has been described by Zimmermann (23). He finds that the plastids show gradual changes in color and size from the margin to the center of the modified areas in *Farfugium grande* and in *Achyranthes Verschaffeltii*. Küster (12) has studied the plastids in the discolored areas of *Pelargonium zonale*, *P. peltatum*, *Spiraea Bumalda*, and *Ligustrum ovalifolium*, and states that they are incompletely developed or have become bleached and degenerate, apparently through agencies within the cells. Stout (21) states for *Coleus*, "Plastids are present in both green and yellow cells but in yellow cells they are fewer in number, smaller in size and somewhat distorted in shape . . . in extreme cases of yellow development nearly all the cells fail in the production of chlorophyll."

Woods (22) holds that in such cases of variegation the chlorophyll is destroyed by oxidizing enzymes, oxidases and peroxidases, which are normally present in all green plants and under certain conditions may be produced in abnormally large quantities. Woods believes that tobacco mosaic is due to an excess of oxidizing enzymes rather than a *contagium vivum fluidum* as described by Beijerinck.

Very suggestive work has been done by Liesegang (14) Küster (11) and Gebhardt (9), who point out the similarity between the Liesegang patterns and the white and green markings in certain variegated leaves. Küster filled a glass tube with 1/10 per cent gelatine chromate and inserted one end into an 80 per cent solution of silver nitrate. A series of alternate horizontal dark and light zones were formed, which, though the distances between the rings are very short, suggest the green and white zones in such plants as *Scirpus zebrinus*. Instead of simply depositing a drop of the nitrate in one place, various lines and figures may be drawn, and thus many leaf patterns may be imitated. Gebhardt has imitated butterfly wing patterns by arranging the solutions so that the resulting concentric zones formed similar figures.

I have studied conditions of the plastids in the cells at the margins of the chlorotic areas in a number of variegated plants which are classed by Baur as non-infectious, inheritable types. As it seemed desirable to observe the cells in as near their natural condition as possible, sections from 7 to 10 microns in thickness were cut with the aid of the freezing microtome and mounted in water. These sections were cut so that they included both normal green and abnormal white or yellow cells, and of course the more critical marginal cells lying between these two extremes.

Dieffenbachia Seguine Schott has large thick green leaves that are profusely blotched with irregularly shaped, white to yellow spots of various sizes. Excellent colored illustrations of the leaves may be found in Lowe's "Beautiful Leaved Plants." In the pale areas of the leaves there is a gradual decrease in the size of the chloroplasts as the chlorotic areas are approached. Figure 1 shows a typical cell from the normal green tissue; the chloroplasts are deep green, plump, and average from 8 to 10 microns in diameter. In figure 2 is shown a cell which was selected from those exhibiting the first decided changes from the normal. The smallest chloroplast in this cell measured 3 microns while the average size was 6 microns in length. A few of the plastids were apparently like the normal ones in healthy cells. Figure 3 represents a cell which was selected from the marginal cells which were about midway between the white and green tissues, and which showed a still greater decrease in size, color, and in the number of the chloroplasts, which were also considerably distorted. Figure 4 represents a cell that is almost colorless, showing only a few highly refractive pale green amorphous masses, which appear to be disorganized chloroplasts. At the base of the cell may be seen three pale green bodies which still retain a resemblance to chloroplasts, but they are very small and lack the amount of chlorophyll found in healthy plastids. Figure 5 shows a section through the critical zone. At the extreme right may be seen healthy cells with large, plump, normal plastids. Towards the left we see a gradual decrease in number, size, and color of the plastids until cells showing no green are reached. In these latter there is no evidence of any chlorophyll bearing bodies. The nuclei so far as I am able to determine are like any other resting nuclei and show no modifications in size, shape or general appearance.

Leaves of *Dracaena Godseffiana* Sander have irregularly distributed yellow to white rounded spots which vary in size. Sections through a yellow spot show that there are no chloroplasts in the abnormal tissue. The marginal cells from such a spot show from one to several plastids, which are irregular in shape, somewhat smaller, and contain less chlorophyll than the normal ones in the green tissue (FIG. 7a). Here and there in certain marginal cells chloroplasts can be found that show no visible differences from the normal (FIG. 7b). The general tendency of the plastids in the marginal cells is to be on that side of the cell which is nearest the normal green tissue, though they frequently may be found on the opposite side. The transition from normal green to colorless in this case is more abrupt than in *Dieffenbachia*, but is not absolutely sharp and definite, since the marginal cells show both normal and abnormal chloroplasts.

The rounded yellow spots of the leaf of *Ligularia Kaempferi* Sieb. & Zucc. (*Farfugium grande* Lindl.) show a gradual diminution in the amount of green color from the solid green cells at the border to the almost colorless central tissue. The areas consist visibly of more or less definite concentric zones, which range from normal green through various tones of green to yellow, and in the larger spots to white. This pattern is very suggestive of the Liesegang rings referred to above. The solid green parts of the leaf, on examination, present a characteristic marbled appearance due to variations in color. At first sight this would seem to be due to differences in the thickness of the leaf, since most of the deeper shades of green are near the veins, but closer examination reveals variation in color of the vein islets, and sections show that the leaf is actually of uniform thickness in the marbled areas. Sections of fresh material show no perceptible differences in the number, size and form of the chloroplasts in the darker and lighter parts of the leaf, and the cells are in general apparently normal. A slight variation in the intensity of the green color of the plastids may be detected, and it is to this that the marbled appearance is due. On the very margin of the modified spot the chloroplasts appear normal except in color, which is a little paler than the normal green, and four or five cells farther away from the margin there is a gradual falling off in the intensity of color until a delicate tint of green is reached, accompanied by a slight irregularity in form and size

of the plastid. From this point on the plastids become irregular in form and become more and more disorganized until they appear in the cells of the white tissue as colorless, amorphous masses. Figure 6 shows a relatively short transition zone between the white and green. In the middle of the spot there are no visible chloroplasts.

The chloroplasts in the pale zones of the sedge *Scirpus zebrinus* show degenerative changes similar to those described for *Dieffenbachia* and *Dracaena*. There are no plastids in the middle of the colorless areas, and a few abnormal ones in the marginal cells.

Other variegated leaves studied, from species of *Croton*, *Abutilon*, and *Plantago lanceolata*, showed practically the same gradual degeneration of the plastids on the margins of the discolored areas as in the above described plants.

In all these cases it would seem that the spotting must be caused by a chlorophyll destroying agent which spreads radially and disorganizes the plastids as it penetrates from cell to cell. The possible nature and origin of such a virus or toxic agent is generally admitted to be as yet quite obscure.

The variegated form of *Oplismenus compositus* Beauv. (*Panicum variegatum* Hort.) shows much more marked differences in the cells of the green and white areas. In the white areas the mesophyll is almost completely atrophied, and the epidermal cells are hypertrophied to the extent that the thickness of the leaf in this region, even if there is no mesophyll tissue, is equal to the thickness of the green part of the leaf, in which there are regularly two layers of mesophyll cells. The two layers of the normal mesophyll consist of a palisade layer which is not much differentiated, and one other mesophyll layer. The chloroplasts are large, deep green and plump. The average thickness of the green portion of the leaves is about 100 microns in the lamina between the veins. The upper epidermal cells average 32 microns in thickness, the lower epidermal cells are somewhat thicker, while the two green mesophyll layers together have an average thickness of 32 microns (FIG. 9). In the colorless areas the thickness of the leaf is about the same as that of the normal green bands, but, as stated, there is practically no chlorophyll bearing mesophyll, the space between the epidermal layers is reduced to negligible dimensions, and in many places the epidermal layers are so hypertrophied as to completely fill the

space between them. The lower epidermal cells measure from 25 to 30 microns in the white areas (FIG. 8). Where the upper and lower epidermal layers do not touch there are sometimes very small cells to be seen, which may contain a plastid or two, or there may be simply large or small intercellular spaces. Sections of the leaves of the green form of this grass show a structure which is identical with the green regions of the variegated form. It is of great interest in this case to know whether the mesophyll is hypoplastic, there being an inhibition of cell division, or whether the mesophyll cells are formed and then atrophy. I am continuing my study of this form and certain others.

This work has been done under the direction of Professor R. A. Harper, and it is a pleasure to record my indebtedness for his stimulating suggestions during its progress, and for his valuable criticisms in the preparation of this paper.

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Explanation of plate 17

FIG. 1. DIEFFENBACHIA SEGUINE: an apparently normal cell from the green portion of the leaf.

FIG. 2. Cell from extreme margin of a white spot. Plastids are smaller and have fallen off slightly in color.

FIG. 3. Cell from marginal area and nearer the white than the foregoing. Plastids are still paler and tend to be slightly distorted.

FIG. 4. Cell showing only three very pale irregular chloroplasts at the bottom, and two disorganized masses of pale green material, evidently degenerate chloroplasts, at *a* and *b*.

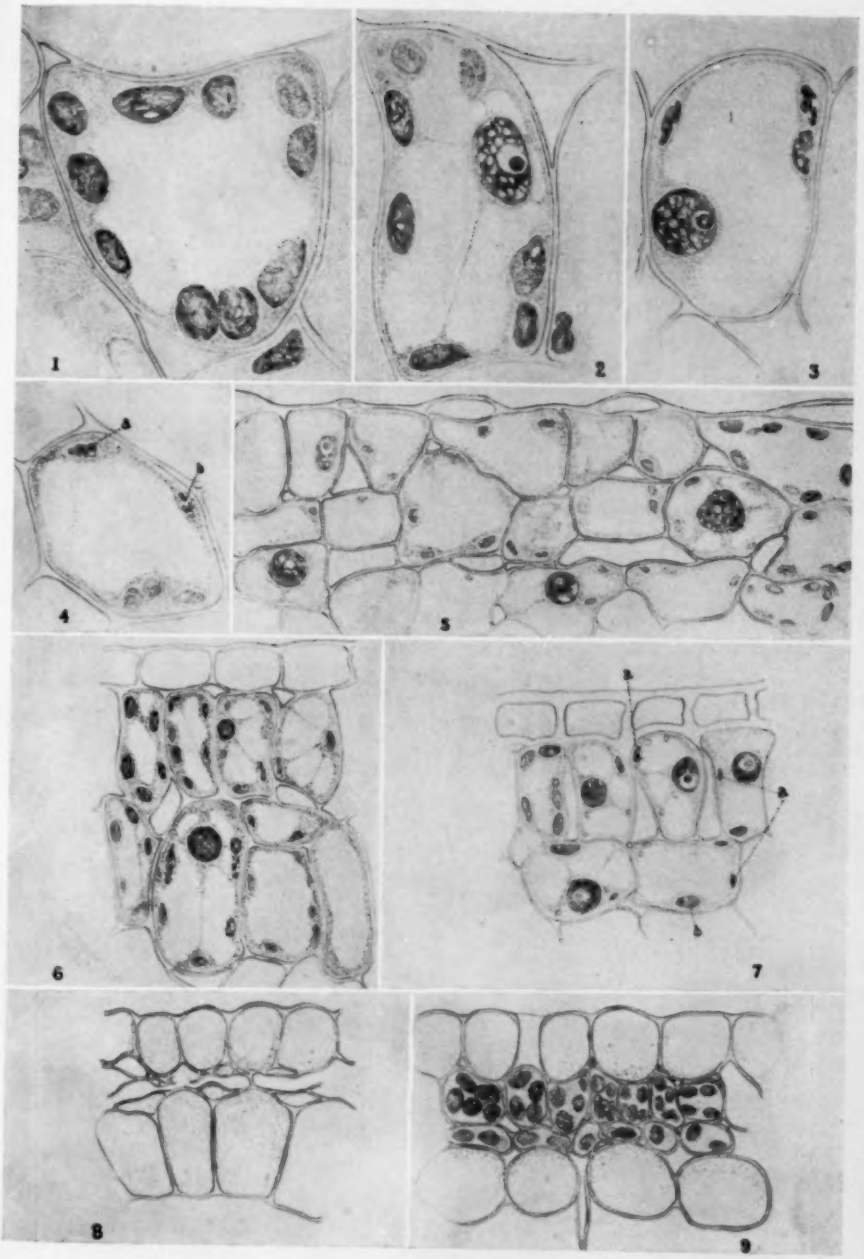
FIG. 5. Section through the marginal area showing apparently normal green cells at the extreme right. To the left the cells show successive stages in the degeneration of the plastids.

FIG. 6. LIGULARIA KAEMPFERI (*Farfugium grande*): cells from marginal area of yellow spot. Upper left cell normal; other cells show various stages of degenerating chloroplasts.

FIG. 7. DRACAENA GODSEFFIANA: upper left is normal cell. At *a* are pale irregular plastids. At *b* is an apparently normal plastid in a cell showing degenerated plastids.

FIG. 8. OPLISMENUS COMPOSITUS (*Panicum variegatum*): cells from white area, showing hypertrophied epidermal cells and absence of mesophyll.

FIG. 9. Section through the green area; mesophyll present and plastids green and plump. FIGS. 1–4, × about 770; FIGS. 5, 6, 7, × about 365; FIGS. 8, 9, × 400.



HEIN: PLASTIDS IN VARIEGATED PLANTS

UNIV. OF MICH.

INDEX TO AMERICAN BOTANICAL LITERATURE
1904–1925

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Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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